IDENTIFICATION OF A LARGE NUMBER OF BIOLOGICAL (MICRO)ORGANISMS GROUPS AT DIFFERENT LEVELS BY THEIR DETECTION ON A SAME ARRAY

Cross-Reference to Related Applications

[0001] This is a continuation-in-part of U.S. Patent Application No. 09/817,014 filed March 23, 2001, which claims priority to European Application Serial Number 00870055.1 filed on March 24, 2000, and European Application Serial Number 00870204.5 filed on September 15, 2000, the disclosures of which are incorporated herein by reference in their entireties.

Background of the Invention

Field of the Invention

[0002] The present invention relates to the diagnosis and analytical assays and is related to a method and kit comprising reagents and means for the identification, detection and/or quantification of a large number of (micro)organisms of different groups (classes, family, genus, species, individual among other ones) by their identification or the identification of a component thereof on a same array.

[0003] The invention is especially suited for the simultaneous identification and/or quantification of groups and sub-groups of (micro)organisms or related genes present in the same biological sample.

[0004] The present invention also provides a two step method for detecting first for the presence of any of the search (micro)organisms followed by its identification.

Description of the Related Art

[0005] Identification of an organism or microorganisms can be performed based on the presence in their genetic material of specific sequences. Identification of a specific organism can be performed easily by amplification of a given sequence of the organism using specific primers and detecting or identifying the amplified sequence.

[0006] However, in many applications especially in diagnostic, possible organisms present in biological samples are numerous and belong to different families, genus, species, subspecies or even individuals. Amplifications of each of the possible organisms is difficult and expensive. A simple method is thus required for such multi-parametric, multi-levels analysis.

[0007] Amplification of a given sequence is performed by several methods such as the polymerase chain reaction (PCR) (US patents 4,683,195 and 4,683,202), ligase chain reaction (LCR) (Wu and Wallace, 1989, Genomics 4: 560-569) or the Cycling Probe Reaction (CPR) (US patent 5,011,769) which are the most common. One particular way to detect for the presence of a given sequence and thus of a particular organism is to follow the appearance of amplicons during the amplicon cycles. The method is called the real time PCR. A fluorescent signal appears when the amplifications are formed and the amplification is considered as positive when reaching a threshold.

[0008] Detecting the amplicons can also be performed after the amplification by methods based on the specific recognition of amplicons to complementary sequences. The first supports used for such hybridization were the nitrocellulose or nylon membranes. However, the methods were miniaturized and new supports such as conducting surfaces, silica, and glass were proposed together with the miniaturization of the detection process. Microarrays or DNA Chips are used for multiple analysis of DNA or RNA sequences either after an amplification step or after a retro-transcription into a cDNA. The target sequences to be detected are labeled during the amplification or copying step and are then detected and possibly quantified on arrays. The presence of a specific target sequence on the arrays is indicative of the presence of a given gene or DNA sequence in the sample and thus of a given organism which may then be identified. The problem of detection becomes difficult when several sequences are homologous to each other, but have to be specifically discriminated upon the same array. This technical problem is the condition to use arrays for many diagnostic purpose since organisms or micro-organisms of interest are often very similar to others on a taxonomic basis and present almost identical DNA sequences.

[0009] The Company Affymetrix Inc. has developed a method for direct synthesis of oligonucleotides upon a solid support, at specific locations by using masks at each step of the processing. Said method comprises the addition of a nucleotides on growing synthesized

oligonucleotides in order to obtain the desired sequences at the desired locations. This method is derived from the photolithographic technology and is coupled with the use of photoprotective groups, which are released before a new nucleotide is added (US-5,510,270). However, only small oligonucleotides are present on the surface, and said method finds applications mainly for sequencing or identifying a pattern of positive spots corresponding to each specific oligonucleotide bound on the array. The characterization of a target sequence is obtained by cutting this polynucleotide into a small oligonucleotides and comparison of the hybridization pattern with a reference sequence. Said technique was applied to the identification of Mycobacterium tuberculosis rpoB gene (WO 97/29212), wherein the capture nucleotide sequence comprises less than 30 nucleotides and from the analysis of two different sequences that may differ by a single nucleotide (the identification of SNPs or genotyping). Small capture oligonucleotide sequences (having a length comprised between 10 and 20 nucleotides) are preferred since the discrimination between two oligonucleotides differing in one base is higher, when their length is smaller.

[0010] The method is complicated by the fact that it cannot directly detect amplicons resulting from genetic amplification (PCR). A double amplification is performed with primer(s) bearing a T3 or T7 sequences and then a retrotranscription with a RNA polymerase. These RNA are cut into pieces of about 40 bases before being detected on an array (example 1 of WO 97/29212). Each sequence requires the presence of 10 capture nucleotide sequences and 10 control nucleotide sequences to be identified on the array. The reason for this complex procedure is that long DNA or RNA fragments hybridize very slowly on small oligonucleotide capture nucleotide sequences present on the surface. Said methods are therefore not suited for the detection of homologous sequences, since the homology varies along the sequences and so part of the pieces will hybridize on the same capture nucleotide sequences. Therefore, a software for the interpretation of the results is incorporated in the method for allowing interpretation of the obtained data. The main reason not to perform a single hybridization of the amplicons on the array is that the amplicons will rehybridize in solution much faster than hybridize on the small capture nucleotide sequences of the array.

[0011] One consequence of such constraints is that polynucleotides are analyzed on oligonucleotides based arrays, only after being cut into oligonucleotides. For gene

expression array which is based on the detection of cDNA copy of the mRNA, the problem still exist but is less acute since the cDNA is single stranded. The fragments are also cut into smaller species and the method requires the use of several capture oligonucleotide sequences in order to obtain a pattern of signals which attest the presence of a given gene. Said cutting also decreases the number of labeled nucleotides, and thus reduces the obtained signal. In the case of cDNA analysis, the use of long capture polynucleotide sequences gives a much better sensitivity to the detection. In many gene expression applications, the use of long capture nucleotide sequences is not a problem, when cDNAs to be detected originate from genes having different sequences, since the difference in the sequence is sufficient in order to avoid cross reactions between them even on a sequence longer than 100 bases so that polynucleotides can be used as capture nucleotide sequences. Long capture nucleotide sequences give the required sensitivity but they will hybridize to other homologous sequences.

[0012] The detection of Single Nucleotide Polymorphism in the DNA is just one particular aspect of the detection of homologous sequences. The use of arrays has been proposed to discriminate two sequences differing by one nucleotide at a particular location of the sequence. Since DNA or RNA sequences are in low copy numbers, their sequences are first amplified so that double stranded sequences are analyzed on the array. Several methods have been proposed to detect such a base change in one location. The document WO 97/31256 proposes the use of two oligonucleotide sequences : the first one with a part specific and a part addressable, the second one with a part specific and a part labeled. After ligation in solution, the product is immobilized on an array with capture nucleotide sequences with a least a part complementary of the addressable part. The detection of SNP is the basis for polymorphism determination of individual organism, but also for its genotyping, since the genome of individuals differ from each other in the same species or subspecies by said SNPs. The presence of particular SNP affect the activities of enzymes like the P450 and make them more or less active in the metabolism of a drug.

[0013] The capture oligonucleotide present on the array can also be used as primers for extension once the target nucleotide hybridized. The document WO 96/31622 proposes to identify a nucleotide at a given location upon a sequence by elongation of a capture nucleotide sequence with detectable modified nucleotides in order to detect the

given spots, where the target has been bound with the last nucleotide of the capture nucleotide sequence being complementary of a target sequence at this particular position. The document WO 98/28438 proposes to complete several cycles of hybridization-elongation steps to label a spot in order to compensate for a low hybridization yield of the target sequence. This method allows identification of a nucleotide at a given location of a sequence by labeling of a spot of the elongated capture nucleotide sequence.

[0014] Prior to elongation, the capture nucleotide sequences present on the array can be digested by a nuclease in order to differentiate between matched and the unmatched heteroduplexes (US-5,753,439). Use of nuclease for identification of sequences has also been proposed (EP 0721016). A second labeled nucleotide sequence complementary of the targets has also been proposed to be added to the hybridized targets and being ligate to the capture nucleotide sequence if the last nucleotide of the targets is complementary to the targets a this position (WO 96/31622).

[0015] The document EP-0785280 proposes a detection of polymorphism based on the hybridization of the target nucleotides on blocks containing several oligonucleotide sequences differing by one base each and obtain a ratio of intensity for determining which sequences are the perfect hybridization matches.

[0016] Using membranes or nylon supports are proposed to increase the sensitivity of the detection of polynucleotides on solid support by incorporation of a spacer between the support and the capture nucleotide sequences. Van Ness et al. (Nucleic Acids Research, vol. 19, p.3345, 1991) describe a poly(ethyleneimine) arm for the binding of DNA on nylon membranes. The document EP-0511559 describes a hexaethylene glycol derivative as spacer for the binding of small oligonucleotides upon a membrane. When membranes like nylon are used as support, there is no control of the site of binding between the solid support and the oligonucleotides and it was observed that a poly dT tail increased the fixation yield and so the resulting hybridization (WO 89/11548).

[0017] Guo et al. (Nucleic Acids Research 22, 5456, 1994) teach the use of poly dT of 15 bases as spacer for the binding of oligonucleotides on glass with increased sensitivity of hybridization.

[0018] The publication of Anthony et al. (Journal of clinical microbiology, vol. 38:2, p.7817-8820) describes the use of a membrane array for the detection of 23 S ribosomal

DNA of various bacterial species after PCR amplification. Targets to detect are rDNA amplified from bacteria by consensus PCR and the detection is obtained on nylon array containing capture nucleotide sequences for said bacteria and having the capture nucleotide sequences having between 20 and 30 bases which are covalently linked to the nylon, and there is no control of the portion of the sequence which is available for hybridization. rDNA are multi-copies DNA which are used in order to compensate for the low detection yield of the method. Also, because of the use of small capture nucleotide sequences they can only detect individual bacterial species by their specific sequence and not the family or genus.

[0019] However these patents neither described nor suggested that it is was possible to use a component of a (micro)organism, especially a genetic sequence, to identify said (micro)organism together with the identification of the group to which these (micro)organisms belong. Also there is neither an indication nor a suggestion in the state of the art that polynucleotides can be used as capture sequences in microarrays in order to differentiate a binding between homologous polynucleotides sequences and to permit identification of one target sequence among other species, genus or families of (micro)organisms sequences.

[0020] Also there is no indication nor suggestion that homologous sequences differing by one nucleotide at one location of the sequence (such as observed in polymorphism analysis) could be detected by hybridization of the amplified sequences on corresponding capture nucleotide sequences.

[0021] Prior to the invention, it was unknown that it is possible to identify in a two step process, i.e. an amplification followed by a direct hybridization of the amplicons on an array, organisms belonging to the same group, to two groups or more together with the specific identification of the groups as such. Also it was unknown that it was possible to identify organisms belonging to a group and sub-group together with the specific identification of these group and sub-group. Also that such identification could be obtained by using polynucleotide as capture sequences for all detections.

[0022] Also it was unknown that polynucleotides could be used for the identification of homologous polynucleotide sequences differing by one nucleotide present in a particular location of the sequence.

[0023] Also it was unknown that homologous polynucleotide sequences could be discriminated and detected on an array directly after amplification with a very high sensitivity.

Summary of the Invention

[0024] The present invention is premised in part on the discovery that arrays can be used to obtain a discrimination between a homologous (biological) component (such as a genetic sequence) of different (micro)organisms belonging to several groups together with the identification of these groups as such.

[0025] The present invention is especially useful in using arrays to discriminate between homologous genetic sequences (amino acid sequences and nucleotide sequences) belonging to several groups of organisms together with the identification of these groups as such.

[0026] The invention provides a method and a device which are based upon a simplified technology requiring the use of a single or limited number of primer pair(s) in an amplification step to detect the presence of the specific target or group of target sequence(s) and followed by the identification (detection and/or quantification) of said specific target or groups of target genetic sequence(s) by recording in a single spot identification upon said micro-array and in the same experimental protocol, said signal being either specific of the organism or the group or sub-group of organisms.

[0027] The present invention further provides means for an identification of organisms differing by single base difference of a given nucleotide sequence followed by hybridization of their amplified polynucleotide sequences upon arrays.

Brief Description of the Drawings

[0028] Figure 1 is a schematic presentation of the step used in the method of the invention for the identification of 5 *Staphylococcus* species on biochips after PCR amplification with consensus primers.

[0029] Figure 2 represents the design of an array which allows the determination of the 5 most common *Staphylococcus* species, of the presence of any *Staphylococcus* strain and of the MecA gene.

[0030] Figure 3 presents the effect of the length of the specific sequence of a capture nucleotide sequence on the discrimination between sequences with different level of homology.

[0031] Figure 4 shows the sensitivity obtained for the detection of *FemA* sequences from *S. aureus* on array bearing the small specific capture nucleotide sequence for a *S. aureus* and a consensus sequence.

Definitions

[0032] The terms "nucleic acid, oligonucleotide, array, nucleotide sequence, target nucleic acid, bind substantially, hybridizing specifically to, background, quantifying" are the ones described in the international patent application WO 97/27317 incorporated herein by reference. The term polynucleotide refers to nucleotide or nucleotide like sequences of more than 100 bases long.

[0033] The terms "nucleotide triphosphate, nucleotide, primer sequence" are those described in the document WO 00/72018 and WO 01/31055 incorporated herein by references.

[0034] The terms "homologous genetic sequences" mean amino acid or nucleotide sequences having a percentage of amino acids or nucleotides identical at corresponding positions which is higher than in purely random alignments. They are considered as homologous when they show a minimum of homology (or sequence identity) defined as the percentage of identical nucleotides or amino acids found at each position compared to a total of nucleotides or amino acids, after the sequences have been optimally aligned taking into account additions or deletions (like gaps) in one of the two sequences to be compared. Genes coding for a given protein but present in genetically different sources like different organisms are usually homologous. Also in a given organism, genes coding for proteins or enzymes of the same family (Interleukins, cytochrome b, p. 450). The degree of homology (or sequence identity) can vary a lot as homologous sequences may be homologous only in one part, a few parts or portions or all along their sequences. The parts or portions of the sequences that are identical in both sequences are said conserved. Protein domains which present a conserved three dimensional structure are usually coded by homologous sequences and even often by a unique exon. The sequences showing a high

degree of invariance in their sequences are said to be highly conserved and they present a high degree of homology.

[0035] The terms “group, sub-group and sub-sub-group” refer first to the classification of biological organisms in taxas kingdom, branches, classes, orders, families, genus, species, sub-species, varieties or individuals. These constitute different levels of biological taxonomical organization. Groups also refer to organisms which have some aspects in common, but some genetic difference like for example the GMO plants, transgenic or chimeric animals. For the purpose of this invention, the common aspects have to be reflected into common or homology DNA or RNA sequences and the dissimilarities or differences in DNA sequences. Genes sequences can also be classified in groups and sub-group independently of their organism origins and are as such part of the invention. They will then refer to groups or sub-groups of genes which belong to a given family such as the cytochrome P450 genes, the protein kinases, the G receptor coupled proteins and others. These genes are homologous to each other as defined here above.

[0036] Classification of genes (nucleotide sequences) are used as the basis of molecules paleontology for establishing the classification of organisms into species, genus, family, orders, classes branches, kingdom and taxus.

Detailed Description of the Preferred Embodiment

[0037] The present invention is related to an identification and/or quantification method of a biological (micro)organism or a (biological) component thereof, said (micro)organism or its component being possibly present in a sample, preferably a biological sample, among at least two, preferably at least four, other related (micro)organisms or components; said method comprising the step of:

[0038] possibly extracting original components from the (micro)organisms;

[0039] possibly labeling said (micro)organism or its components being target,

[0040] putting into contact the (micro)organism or its components being targets with capture molecules bound to an insoluble support, preferably a non-porous solid support,

[0041] discriminating the binding of said targets, specific of a (micro)organism or its component by detecting, quantifying and/or recording a signal resulting from the specific binding between said targets and their corresponding specific capture molecules; wherein

said capture molecules are bound to an insoluble solid support at a specific location according to an array, said array having a density of at least 4 different bound capture molecules/cm^2 of solid support surface and wherein the binding between the targets and their corresponding capture molecules forms said signal at the expected location, the detection of a single signal allowing a discrimination of a target being specific of said (micro)organism or its components from other related (micro)organisms or other related components.

[0042] Advantageously, said method further comprises the step of identifying and/or quantifying the presence of several groups, subgroups or sub-subgroups of components or (micro)organisms, comprising said components being related to each other until possible individual genetic sequences (nucleotide and/or amino acid sequences) wherein the binding of targets and corresponding specific capture molecules forms a signal at an expected location allowing the identification of a target specific of a group, sub-group or sub-subgroup of components or (micro)organisms comprising said components.

[0043] Therefore, the biological component according to the invention could be a nucleotide sequence specific of a (micro)organism or an amino acid sequence (peptide) specific of a (micro)organism. Examples of said molecules are homologous nucleotide sequences or peptides presenting a high homology such as receptors, HLA molecules, cytochrome P450, etc.

[0044] Furthermore, the inventors have discovered that it is possible to drastically simplify the identification or quantification of one or several (micro)organisms among many other ones present in such biological sample, said identification and/or quantification being obtained by combining a single amplification using common primer pairs and an identification of the possible (micro)organisms by detecting, quantifying and/or possibly recording upon an array the presence of a single signal resulting only between a capture nucleotide sequence and its corresponding target nucleotide sequence and thereafter correlating the presence of said detected target nucleotide sequence to the identification of a nucleotide sequence specific of said (micro)organism(s).

[0045] This means that the method and device according to the invention will allow the easy identification/detection of a specific sequence among other homologous sequences and possibly its quantification (characterization of the number of copies or

presence of said organisms in a biological sample) of a target nucleotide sequence, said target sequence having a nucleotide sequence specific of said (micro)organisms.

[0046] Such identification may be obtained directly, after washing of possible contaminants (unbound sequences), by detecting and possibly recording a single spot signal at one specific location, wherein said capture nucleotide sequence was previously bound and said identification is not a result of an analysis of a specific pattern upon the microarray as proposed in the system of the state of the art. Therefore, said method and device do not necessarily need a detailed analysis of said pattern by an image processing and a software analysis.

[0047] This invention was made possible by discovering that target sequences can be discriminated from other homologous ones upon an array with high sensitivity by using bound capture nucleotide sequences composed of at least two parts, one being a spacer bound by a single and advantageously predetermined (defined) link to the support (preferably a non porous support) and the other part being a specific nucleotide sequence able to hybridize with the nucleotide target sequence.

[0048] Furthermore, said detection is greatly increased, if high concentrations of capture nucleotide sequences are bound to the surface of the solid support.

[0049] The present invention is related to the identification of a target nucleotide sequence obtained from a biological (micro)organism or a portion thereof, especially a gene possibly present in a biological sample from at least 4 other homologous (micro)organisms or a portion thereof, said other (micro)organisms could be present in the same biological sample and have homologous nucleotide sequences with the target.

[0050] Said identification is obtained firstly by a genetic amplification of said nucleotide sequences (target and homologous sequences) by common primer pairs followed (after washing) by a discrimination between the possible different target amplified nucleotide sequences. Said discrimination is advantageously obtained by hybridization upon the surface of an array containing capture nucleotide sequences at a given location, specific for a target nucleotide sequence specific for each (micro)organism to be possibly present in the biological sample and by the identification of said specific target nucleotide sequence through the identification and possibly the recording of a signal resulting from the specific

binding of this target nucleotide sequence upon its corresponding capture nucleotide sequence at the expected location (single location signal being specific).

[0051] According to the invention, the preferred method for genetic amplification is the PCR using two anti-parallel consensus primers which can recognize all said target homologous nucleotide sequences but other genetic amplification methods may be used.

[0052] Therefore, said (micro)organisms could be present in any biological material or sample including genetic material obtained (virus, fungi, bacteria, plant or animal cell, including the human body). The biological sample can be also any culture medium wherein microorganisms, xenobiotics or pollutants are present, as well as such extract obtained from a plant or an animal (including a human) organ, tissue, cell or biological fluid (blood, serum, urine, sputum, etc).

[0053] The method according to the invention can be performed by using a specific identification (diagnostic and/or quantification) kit or device comprising at least an insoluble solid support upon which are bound single stranded capture nucleotide sequences (preferably bound to the surface of the solid support by a direct covalent link or by the intermediate of a spacer) according to an array with a density of at least 4, preferably at least 10, 16, 20, 50, 100, 1000, 4000, 10 000 or more, different single stranded capture nucleotide sequences/cm^2 insoluble solid support surface, said single stranded capture nucleotide sequences having advantageously a length comprised between about 30 and about 600 bases (including the spacer) and containing a sequence of about 3 to about 60 bases, said sequence being specific for the target (which means that said bases of said sequence are able to form a binding with their complementary bases upon the sequence of the target by complementary hybridization). Preferably, said hybridization is obtained under stringent conditions (under conditions well-known to the person skilled in the art).

[0054] In the method and kit or device according to the invention, the capture nucleotide sequence is a sequence having between 16 and 600 bases, preferably between 30 and 300 bases, more preferably between 40 and 150 bases and the spacer is a chemical chain of at least 6.8 nm long (of at least 4 carbon chains), a nucleotide sequence of more than 15 bases or is nucleotide derivative such as PMA.

[0055] The method, kit and device according to the invention are particularly suitable for the identification of a target, being preferably biological (micro)organisms or a part of it, possibly present in a biological sample where at least 4, 12, 15 or even more homologous sequences are present. Because of the high homology, said nucleotide sequence can be amplified by common primer(s) so that the identification of the target nucleotide sequence is obtained specifically by the discrimination following its binding with the corresponding capture nucleotide sequence, previously bound at a given location upon the microarray. The sensitivity can be also greater increased if capture nucleotide sequences are spotted to the solid support surface by a robot at high density according to an array. A preferred embodiment of the invention is to use an amount of capture nucleotide sequences spotted on the array resulting in the binding of between about 0.01 to about 5 pmoles of sequence equivalent/cm^2 of solid support surface.

[0056] The kit or device according to the invention may also incorporate various media or devices for performing the method according to the invention. Said kit (or device) can also be included in an automatic apparatus such as a high throughput screening apparatus for the detection and/or the quantification of multiple nucleotide sequences present in a biological sample to be analyzed. Said kit or apparatus can be adapted for performing all the steps or only several specific steps of the method according to the invention.

[0057] In the method, the kit (device) or apparatus according to the invention, the length of the bound capture nucleotide sequences is preferably comprised between about 30 and about 600 bases, preferably between about 40 and about 400 bases and more preferably between about 40 and about 150 bases. Longer nucleotide sequences can be used if they do not lower the binding yield of the target nucleotide sequences usually by adopting hairpin based secondary structure or by interaction with each other.

[0058] In a preferred embodiment, the specific part of the capture nucleotide sequence is bound onto a nucleotide sequence of between 20 and 600 bases.

[0059] In another preferred embodiment, all capture molecules are polynucleotides of more than 100 base long.

[0060] In another embodiment, the capture nucleotide sequence is linked to a polymer molecule bound to the solid support. The polymer is preferably a chain of at least 10 atoms, selected from the group consisting of poly-ethyleneglycol, polyaminoacids,

polyacrylamide, poly-aminosaccharides, polyglucides, polyamides, polyacrylate, polycarbonate, polyepoxides or poly-ester (possibly branched polymers).

[0061] If the homology between the sequences to be detected is low (between 30 and 60%), parts of the sequence which are specific in each sequence can be used for the design of specific capture nucleotide sequences binding each of the different target sequences. However, it is more difficult to find part of the sequence sufficiently conserved as to design "consensus" sequences which will amplify or copy all desired sequences. If one pair of consensus primers is not enough to amplify all the homologous sequences, then a mixture of two or more primers pairs is added in order to obtain the desired amplifications. The minimum homologous sequences amplified by the same consensus primer is two, nut there is no limitation to said number.

[0062] If the sequences show high degree of homology, higher than 60% and even higher than 90%, then the finding of common sequence for consensus primer is easily obtained, but the choice for specific capture nucleotide sequences become more difficult.

[0063] In another preferred embodiment of the invention, the capture nucleotide sequences are chemically synthesized oligonucleotides sequences shorter than 100 bases (easily performed on programmed automatic synthesizer). Such sequences can bear a functionalized group for covalent attachment upon the support, at high concentrations.

[0064] Longer capture nucleotide sequences are preferably synthesized by (PCR) amplification (of a sequence incorporated into a plasmid containing the specific part of the capture nucleotide sequence and the non specific part (spacer)).

[0065] In a further embodiment of the invention, the specific sequence of the capture nucleotide sequence is separated from the surface of the solid support by at least about 6.8 nm long, equivalent to the distance of at least 20 base pair long nucleotides in double helix form.

[0066] In the method, kit (device) or apparatus according to the invention, the portion(s) (or part(ies)) of the capture nucleotide sequences complementary to the target is comprised between about 3 and about 60 bases, preferably between about 15 and about 40 bases and more preferably between about 20 and about 30 bases. These bases are preferably assigned as a continuous sequence located at or near the extremity of the capture nucleotide sequence. This sequence is considered as the specific sequence for the detection. In a

preferred form of the invention, the sequence located between the specific capture nucleotide sequence and the support is a non specific sequence.

[0067] In another embodiment of the invention, a specific nucleotide sequence comprising between about 3 and about 60 bases, preferably between about 15 and about 40 bases and more preferably between about 20 and about 30 bases is located on a capture nucleotide sequence comprising a sequence between about 30 and about 600 bases.

[0068] The method, kit (device) or apparatus according to the invention are suitable for the detection and/or the quantification of a target which is made of DNA or RNA, including sequences which are partially or totally homologous upon their total length.

[0069] The method according to the invention can be performed even when a target present between an homology (or sequence identity) greater than 30%, greater than 60% and even greater than 80% and other molecules.

[0070] In the method, kit (device) or apparatus according to the invention, the capture nucleotide sequences are advantageously covalently bound (or fixed) upon the insoluble solid support, preferably by one of their extremities as described hereafter.

[0071] The method according to the invention gives significant results which allows identification (detection and quantification) with amplicons in solutions at concentration of lower than about 10 nM, of lower than about 1 nM, preferably of lower than about 0.1 nM and more preferably of lower than about 0.01 nM (= 1 fmole/100 µl).

[0072] Another important aspect of this invention is to use very concentrate capture nucleotide sequences on the surface. If too low, the yield of the binding is quickly lower and is undetectable. Concentrations of capture nucleotide sequences between about 600 and about 3,000 nM in the spotting solutions are preferred. However, concentrations as low as about 100 nM still give positive results in favorable cases (when the yield of covalent fixation is high or when the target to be detected is single stranded and present in high concentrations). Such low spotting concentrations would give density of capture nucleotide sequence as low as 20 fmoles per cm^2. On the other side, higher density was only limited in the assays by the concentrations of the capture solutions, but concentrations still higher than 3,000 nM give good results.

[0073] The use of these very high concentrations and long nucleotide sequences are two unexpected characteristic features of the invention. The theory of DNA

hybridization proposed that the rate of hybridization between two DNA complementary sequences in solution is proportional to the square root of the DNA length, the smaller one being the limited factor (Wetmur, J.G. and Davidson, N. 1968, J. Mol. Biol. 3, 584). In order to obtain the required specificity, the specific sequences of the capture nucleotide sequences had to be small compared to the target. Moreover, the targets were obtained after PCR amplification and were double stranded so that they reassociate in solution much faster than to hybridize on small sequences fixed on a solid support where diffusion is low thus reducing even more the rate of reaction. It was unexpected to observe a so large increase in the yield of hybridization with the same short specific sequence.

[0074] The amount of a target which "binds" on the spots is small compared to the amount of capture nucleotide sequences present. So there is a large excess of capture nucleotide sequence and there was no increase of binding if more capture nucleotide sequences were present.

[0075] One may perform the detection on the full length sequence obtained after amplification or copy and when labeling is performed by incorporation of labeled nucleotides, more markers are present on the hybridized target making the assay sensitive.

[0076] The method, kit and apparatus according to the invention may comprise the use of other bound capture nucleotide sequences, which may have the same characteristics as the previous ones and may be used to identifying a target from another group of homologous sequences (preferably amplified by common primer(s)).

[0077] In the microbiological field, one may use consensus primer(s) specific for each family, or genus, of micro-organisms and then identify some or all the species of these various family in an array by using capture nucleotide sequences of the invention. Detection of other sequences can be advantageously performed on the same array (i.e. by allowing an hybridization with a standard nucleotide sequence used for the quantification, with consensus capture nucleotide sequences for the same or different micro-organisms strains, with a sequence allowing a detection of a possible antibiotic resistance gene by micro-organisms or for positive or negative control of hybridization). Said other capture nucleotide sequences have (possibly) a specific sequence longer than 10 to 60 bases and a total length as high as 600 bases and are also bound upon the insoluble solid support (preferably in the array made with the other bound capture nucleotide sequences related to

the invention). A long capture nucleotide sequence may also be present on the array as consensus capture nucleotide sequence for hybridization with all sequences of the microorganisms from the same family or genus, thus giving the information on the presence or not of a microorganism of such family, genus in the biological sample.

[0078] The same array can also bear capture nucleotide sequences specific for a bacterial group and as specific application to Gram-positive or Gram-negative strains or even all the bacteria.

[0079] Another application is the detection of homologous genes from a consensus protein of the same species, such as various cytochromes P450 by specific capture nucleotide sequences with or without the presence of a consensus capture nucleotide sequence for all the cytochromes P450 possibly present in a biological sample. Such detection is performed at the gene level by retrotranscription into cDNA.

[0080] The solid support according to the invention can be or can be made with materials selected from the group consisting of glasses, electronic devices, silicon supports, plastic supports, silica, metal or a mixture thereof in format such as slides, compact discs, gel layers, microbeads. Advantageously, said solid support is a single glass slide which may comprise additional means (barcodes, markers, etc.) or media for improving the method according to the invention.

[0081] The amplification step used in the method according to the invention is advantageously obtained by well known amplification protocols, preferably selected from the group consisting of PCR, RT-PCR, LCR, CPT, NASBA, ICR or Avalanche DNA techniques.

[0082] Advantageously, the target nucleotide sequence to be identified is labeled previously to its hybridization with the single stranded capture nucleotide sequences. Said labeling (with known techniques from the person skilled in the art) is preferably also obtained upon the amplified sequence previously to the denaturation (if the method includes an amplification step).

[0083] Advantageously, the length of the target nucleotide sequence is selected as being of a limited length preferably between 50 and 2000 bases, preferably between 100 and 400 bases and more preferably between 100 and 200 bases. This preferred requirement depends on the possibility to find consensus primers to amplify the required sequences possibly present in the sample. Too long target nucleotide sequence may reallocate

faster and adopt secondary structures which can inhibit the fixation on the capture nucleotide sequences.

[0084] The amplified target nucleotide sequence can be cut before the hybridization, and the use of one capture sequence for each target sequence to make the interpretation of the results easy.

[0085] The detection of homologous expressed genes is obtained by first retrotranscription of the mRNA by a consensus primer, the preferred one being the poly dT. In one embodiment, the retrotranscribed cDNA is then amplified by consensus primers as described in this invention.

[0086] According to a further aspect of the present invention, the method, kit (device) or apparatus according to the invention is advantageously used for the identification of different *Staphylococcus* species or variant, preferably the *S. aureus, the S. epidermidis, the S. saprophyticus, the S. hominis or the S. haemolyticus* for homologous organs present together or separately in the biological sample, said identification being obtained by detecting the genetic variants of the *FemA* gene in said different species, preferably by using a common locations in the *FemA* genetic sequence (examples 4, 5, 6, 7). In another aspect of the invention, 16 Staphylococcus species could be detected after amplification by the same primers and identification on the array (example 7).

[0087] Preferably, the primer(s) and the specific portions of said *FemA* sequence used for obtaining amplified products are the ones described hereafter in example 2. These primers have been selected as consensus primers for the amplification of the *FemA* genes of all of the 16 *Staphylococcus* tested and they probably will amplify the *FemA* from all other possible *Staphylococcus* species.

[0088] A further aspect of the invention is the detection of Mycobacteria species, the *M. tuberculosis* and other species, preferably the *M. avium, M. gastrii, M. gordonae, M. intracellulare, M. leprae, M. kansasi, M. malmoense, M. marinum, M. scrofulaceum, M. simiae, M. szulgai, M. xenopi, M. ulcerans* (example 8).

[0089] In a further application of the invention, one array can specifically detect amplified sequences from several bacterial species belonging to the same genus (examples 7 and 8) or from several genus like Staphylococcus, Streptococcus, Enterococcus,

Haemophilus (see table 1) or different bacterial species and genus belonging to the Gram-positive bacteria and/or to the Gram-negative bacteria (examples 16 and 22).

[0090] Preferably, the primer(s) and the specific portions of gyrase (sub-unit A) sequences are used for obtaining amplified products. These primers have been selected as consensus primers for the amplification of the gyrase genes of all of the bacteria tested and they probably will amplify the gyrase from many other possible bacteria species and genus and families.

[0091] The invention is particularly suitable for detection of bacteria belonging to at least two of the following genus families: *Staphylococcus, Enterococcus, Streptococcus, Haemolyticus, Pseudomonas, Campylobacter, Enterobacter, Neisseria, Proteus, Salmonella, Simonsiella, Riemerella, Escherichia, Neisseria, Meningococcus, Moraxella, Kingella, Chromobacterium, Branhamella.*

[0092] The array allows to read the MAGE number by observation of the lines positive for signal bearing the specific capture nucleotide sequences.

[0093] The same application was developed for the G Protein Coupled Receptors (GPCR). These receptors bind all sort of ligands and are responsible for the signal transduction to the cytoplasm and very often to the nucleus by modulating the activity of the transcriptional factors. Consensus primers are formed for the various subtypes of GPCR for dopamine and for serotonin and histamine. The same is possible for the histamine and other ligands.

[0094] The detection of the various HLA types is also one of the applications of the invention. HLA are homologous sequences which differ from one individual to the other. The determination of the HLA type is especially useful in tissue transplantation in order to determine the degree of compatibility between the donor and the recipient. It is also a useful parameter for immunization. Given the large number of subtypes and the close relation between the homologous sequences it was not always possible to perfectly discriminate one sequence among all the other ones and for some of them there was one or two cross-reactions. In this case, a second capture nucleotide sequence complementary to another location of the amplified sequence was added on the array, in order to make the identification absolute.

[0095] Genetic sequences code for proteins so that homologous DNA sequences correspond to homologous amino acid sequences of the encoded proteins while variation in the DNA sequences correspond to variation in amino acid sequence. One embodiment of this invention is to use antibodies for specific capture of proteins from a sample in order to identify the protein and so the organism from which it originates. By choosing appropriate antibodies, the organisms or the group to which it belongs is determined. The HLA typing is given as example of the use of specific antibodies for discriminating the various HLA-A proteins on an array (example 23).

[0096] Discrimination of the Cytochrome P450 forms is one particular application of the invention (example 14).

[0097] The detection of polymorphism sequences (which can be considered as homologous even if differing by only one base) can be made also by the method according to the invention. This is especially useful for the Cytochrome P450 since the presence of certain isoforms modifies the metabolism of some drugs. The invention was found particularly useful for discriminating between the isoforms of Cyto P450 2D6 and 2C19. More generally the invention is particularly well adapted for the discrimination of sequences differing by one base mutation or deletion called Single Nucleotide Polymorphism (SNP). The originality of the invention is to perform the hybridization step directly on the amplified sequences without the necessity to copy into RNA and to cut them into pieces.

[0098] Furthermore, one array can specifically detect amplified sequences from several animal species and genus belonging to several families like Galinacea, Leporidae, Suidae and Bovidae (table 2).

[0099] One array can specifically detect amplified sequences from several fishes species, such as G. morhua, G. macrocephalus, P. flesus, M. merluccius, O. mykiss, P. platessa, P. virens, S. salar, S. pilchardus, A. thazard, T. alalunga, T. obesus, R. hippoglossoides, S. trutta, S. sarda, T. thynnus, S. scombrus belonging to several genera such as Auxis, Sarda, Scomber, Thunnus, Oncorhynch, Salmo, Merluccius, Pleuronectes, Platichtlys, Reinhardtius, Pollachius, Gadus, Sardina, from several families such as Scombridae, Salmonidae, Merluccidae, Pleuronectidae, Gadidae and Clupeidae. (Table 3) Other homologous sequences allow the determination of plant species and genus such as

Potato, tomato, oryza, zea, soja, wheat, barley, bean, carrot belonging to several families (example 19).

[0100] According to a further aspect of the present invention, the method, kit (device) or apparatus according to the invention is advantageously used for the identification of the origin of meat (table 2).

[0101] Preferably, the primer(s) and the specific portions of cytochrome b sequences are used for obtaining amplified products are the ones described hereafter in example 3. These primers have been selected as consensus primers for the amplification of the cytochrome B genes of all of animals tested and they probably will amplify the cytochrome B from many other animals species, genus and families.

[0102] According to a further aspect of the present invention, the method, kit (device) or apparatus according to the invention is advantageously used for the identification of the origin of fishes (table 3).

[0103] Preferably, the primer(s) and the specific portions of said cytochrome b sequences used for obtaining amplified products are the ones described hereafter in example 18. These primers have been selected as consensus primers for the amplification of the cytochrome B genes of all of fishes tested and they probably will amplify the cytochrome B from many other fishes species, genus and families.

[0104] According to a further aspect of the present invention, the method, kit (device) or apparatus according to the invention is advantageously used for the identification of the origin of plants.

[0105] Preferably, the primer(s) and the specific portions of said sucrose synthase sequences used for obtaining amplified products are the ones described hereafter in the examples. These primers have been selected as consensus primers for the amplification of the sucrose synthase genes of all of plants tested and they probably will amplify the sucrose synthase from many other plants species, genus and families.

[0106] According to a further aspect of the present invention, the method, kit (device) or apparatus according to the invention is advantageously used for the identification of the Genetically Modified Organism (GMO). The GMO are produced by insertion into the genome of an organism of one or several external genes together with other regulating or construction sequences.

[0107] Preferably, the primer(s) and the specific portions of said sucrose synthase sequences used for obtaining amplified products are the ones described hereafter in the examples. These primers have been selected as consensus primers.

[0108] Homologous DNA or RNA sequences lead to the expression in cells or tissues of proteins which are also homologous to each other. Therefore, a target component to be detected may be protein which is related to other homologous ones which could be present in the same biological sample. Related proteins means proteins which have some part(s) of their sequence or conformation in common, while said proteins present other part(s) which are specific or the (micro)organisms or a part of said (micro)organisms from which they originate.

[0109] Part or portion of the amino acid sequences are identical between proteins from the same group while other portions are specific of the target to be identified and possibly quantified. Said amino acid sequences present linear or conformational epitopes which can be recognized by specific (monoclonal) antibodies. The discrimination between said specific related targets is possible by specific antibodies or reconstructed antibodies like proteins bearing hypervariable portions of these antibodies. An identification of said common homologous sequences is also possible by using antibodies directed against the common sequence. Therefore, a discrimination between groups, subgroups, sub-subgroups and individual proteins can be made in a single experiment.

[0110] Preferably, antibodies are bound to the solid support as array and are used for the specific capture of the target's components to be identified. For HLA identification, proteins are classified in class I, II and III antigens. The class I is divided into the HLA-A, B, C, E, F and G. Each of them being subdivided into HLA types and subtypes as given in the databank IMGT/HLA. There are more than 476 different alleles of the class I HLA antigens. The heavy chains of the HLA complex of type I possess regions as the $\alpha 1$ and $\alpha 2$ domains which are very polymorphic while other parts as the $\alpha 3$ is more conserved (Auffray and Strominger, 1986, Advanced Hum. Genet. 15, 197). The class II is divided into the HLA-DR, HLA-DP and HLA-DQ. There are more than 430 alleles of the HLA class II. Each type is subdivided into subtypes and sub-subtypes which can be discriminated according to the present invention (example 23).

[0111] In one of the aspects of the invention, typing of Cytochrome P450 proteins is performed using the antibodies directed against cytochrome P450 1A1, 1A2, 2A6, 2C11, 3A4, 4A. These antibodies are available from ABR (Golden, Co, USA).

[0112] According to a further aspect of the present invention, the method, kit (device) or apparatus according to the invention is advantageously used for the identification of the organisms or part of it as provided in the examples cited here above and also the ones presented in the examples 1 to 23.

[0113] Another aspect of the present invention is related to any part of biochips or microarray comprising said above described sequences (especially the specific capture nucleotide sequence described in the examples) as well as a general screening method for the identification of a target sequence specific of said microorganisms of family type discriminated from homologous sequences upon any type of microarrays or biochips by any method.

[0114] After hybridization on the array, the target sequences can be detected by current techniques. Without labeling, preferred methods are the identification of the target by mass spectrometry now adapted to the arrays (US-A-5,821,060) or by intercalating agents followed by fluorescent detection(WO 97/27329).

[0115] The labeled associated detections are numerous. A review of the different labeling molecules is given in W0 97/27317. They are obtained using either already labeled primer or by incorporation of labeled nucleotides during the copy or amplification step. A labeling can also be obtained by ligating a detectable moiety onto the RNA or DNA to be tested (a labeled oligonucleotide, which is ligated, at the end of the sequence by a ligase). Fragments of RNA or DNA can also incorporate labeled nucleotides at their 5' -OH or 3' -OH ends using a kinase, a transferase or a similar enzyme.

[0116] The most frequently used labels are fluorochromes like Cy3, Cy5 and Cy7 suitable for analyzing an array by using commercially available array scanners (General Scanning, Genetic Microsystem,...). Radioactive labeling, cold labeling or indirect labeling with small molecules recognized thereafter by specific ligands (streptavidin or antibodies) are common methods. The resulting signal of target fixation on the array is either fluorescent, colorimetric, diffusion, electroluminescent, bio- or chemiluminescent, magnetic, electric like impedometric or voltametric (US-A-5,312,527). A preferred method is based upon the use of

the gold labeling of the bound target in order to obtain a precipitate or silver staining which is then easily detected and quantified by a scanner.

[0117] Quantification has to take into account not only the hybridization yield and detection scale on the array (which is identical for target and reference sequences) but also the extraction, the amplification (or copying) and the labeling steps.

[0118] The method according to the invention may also comprise means for obtaining a quantification of target nucleotide sequences by using a standard nucleotide sequence (external or internal standard) added at known concentration. A capture nucleotide sequence is also present on the array so as to fix the standard in the same conditions as said target (possibly after amplification or copying); the method comprising the step of quantification of a signal resulting from the formation of a double stranded nucleotide sequence formed by complementary base pairing between the capture nucleotide sequences and the standard and the step of a correlation analysis of signal resulting from the formation of said double stranded nucleotide sequence with the signal resulting from the double stranded nucleotide sequence formed by complementary base pairing between capture nucleotide sequence(s) and the target in order to quantify the presence of the original nucleotide sequence to be detected and/or quantified in the biological sample.

[0119] Advantageously the standard is added in the initial biological sample or after the extraction step and is amplified or copied with the same primers and/or has a length and a GC content identical or differing from no more than 20% to the target. More preferably, the standard can be designed as a competitive internal standard having the characteristics of the internal standard found in the document WO 98/11253. Said internal standard has a part of its sequence common to the target and a specific part which is different. It also has at or near its two ends sequences which are complementary of the two primers used for amplification or copy of the target and similar GC content (WO 98/11253). In the preferred embodiment of this invention, the common part of the standard and the target, means a nucleotide sequence which is homologous to all target amplified by the same primers (i.e. which belong to the same family or organisms to be quantified).

[0120] Preferably, the hybridization yield of the standard through this specific sequence is identical or differ no more than 20% from the hybridization yield of the target sequence and quantification is obtained as described in WO 98/11253.

[0121] Said standard nucleotide sequence, external and/or internal standard, is also advantageously included in the kit (device) or apparatus according to the invention, possibly with all the media and means necessary for performing the different steps according to the invention (hybridization and culture media, polymerase and other enzymes, standard sequence(s), labeling molecule(s), etc.).

[0122] Advantageously, the solid support of the biochips also contain spots with various concentrations (i.e. 4) of labeled capture nucleotide sequences. These labeled capture nucleotide sequences are spotted from known concentrations solutions and their signals allow the conversion of the results of hybridization into absolute amounts. They also allow to test for the reproducibility of the detection.

[0123] The solid support of the biochips can be inserted in a support connected to another chamber and automatic machine through the control of liquid solution based upon the use of microfluidic technology. By being inserted into such a microlaboratory system, it can be incubated, heated, washed and labeled by automates, even for preliminary steps (like extraction of DNA, genetic amplification steps) or the identification and discrimination steps (labeling and detection). All these steps can be performed upon the same solid support.

[0124] The present invention is also related to a method to identify homologous sequences (and the groups to which they belong and eventually the organisms and their groups) possibly present in a biological sample by assay of their genetic material in a array-type format. The method is well adapted for determination of organisms belonging to several groups being themselves members of a super-group. The method is for example well adapted for a biological determination and/or classification of animals, plants, fungi or micro-organisms.

[0125] The method involves the use of multiple capture nucleotide sequences present as arrays, the capture of the corresponding target sequences and their analysis and possibly their quantification. The method also allows the identification of these organisms and their groups by characterization of the positive area of the arrays bearing the required capture nucleotide sequences. One particular specification of the invention being that a positive hybridization resulting in one spot on the array, gives the necessary information for

the identification of the sequence or the organism or the group or sub-group from which it belongs by the person skilled in the art.

[0126] It also provides a method for sequential analysis of the presence of any researched organisms during the genetic amplification followed by the detection of amplicons on the array and identification of the corresponding organisms or groups thereafter.

[0127] Furthermore, the inventors have discovered that is possible to obtain by the method of the invention a very quick and easy identification of such multiple sequences belonging to several groups or sub-groups or sub-sub-groups of sequences being homologous to each others, until possible individual sequences, by combining a single nucleotide amplification, preferably by PCR, using common primer pair(s) together with an identification of the organisms at different level(s) by detecting and possibly recording upon an array having at least 5 different bound single stranded capture nucleotide sequences/cm^2 of solid support surface, the presence of a single signal resulting from the binding between a capture sequence and its (or their) corresponding target sequence(s) and thereafter correlating the presence of said detected target sequences to the identification of a specific genetic sequence among the other ones. The method is especially well adapted for the identification of organism species, genus and family through the analysis of a given part of their genome or gene expressed, these sequences being homologous to each other in the different organisms.

[0128] A single signal means a signal which by itself is sufficient to identify one or more target nucleotide sequence(s) to which it is designed and therefore to give (if necessary) an unambiguous response for the presence or not of the organisms or groups of organism present in the sample or the organisms or group of organisms from which said sample has been obtained.

[0129] The method and device according to the invention allows easy identification/detection of a specific nucleotide sequences among other possible amplified nucleotide sequences and possibly their quantification (characterization of the number of copies or presence of said organisms in a biological sample) of target sequences, said target nucleotide sequences having a nucleotide sequence specific of said organisms or groups of organisms.

[0130] The array may contain capture nucleotide sequences from several organism genus and from several of these genus species. The capture nucleotide sequences may detect the genus, the species and also the family(ies) to which these genus belong. The capture nucleotide sequences may also detect the sub-species and even the individual organisms of one or several species. Individual organisms of a given species are considered as having very homologous sequences differing mainly by single bases within some of their DNA sequences or genes. Homology is important for getting consensus primers and a single base change is sufficient to obtain a discrimination between two target amplicons. If not completed, the discrimination can be confirmed by the use of second capture nucleotide sequences present upon the array and able to bind a same amplicon at different sequence location.

[0131] Said identification is obtained firstly by a genetic amplification of said nucleotide sequences (target sequences) by common primer pair followed (after washing) by a discrimination between the possible different targets amplified according to the above described method.

[0132] The amplified sequences may belong to the same gene, may be part of the same DNA locus and are homologous to each others.

[0133] The method according to the invention further comprises the step of correlating the signal of detection (possibly recorded) to the presence of :

[0134] specific organism(s) groups

[0135] specific organism(s) sub-groups until the possible individuals,

[0136] genetic characteristics of a sequence from a organism,

[0137] polymorphism of said sequence,

[0138] genotyping of organisms based on differences in DNA or RNA sequences

[0139] diagnostic predisposition or evolution (monitoring) of genetic diseases, including cancer of a patient (including the human) from which the biological sample has been obtained.

[0140] The method also applies to the identification and possibly characterization of nucleotide sequences as such independently of the organism. Genes or DNA sequences can be classified in groups and sub-groups and sub-sub-groups according to their sequence homology. Bioinformatic programs exist for sequence alignment and

comparison (such as Clustal, Intelligenetics, Mountain View, California, or GAP, BESTFIT, FASTA and TFASTA in the Wisconsin Genetics Software Package, Genetics computer Group Madison, Wisconsin, USA or Boxshade). A classification can be made according to the percentage of homology and alignment of the sequences. An interest in detection and identification of the sequences from a given family in a given organism, tissue or cell is for example the possibility to detect the effect of any given molecules, biological or pathological conditions (by proteomics, functional genomics, etc.) upon both the overall and the specific genes of one or several families.

[0141] The inventors also find that sensitivity of the assay was increased by using high density of capture nucleotide sequences fixed on the support, being preferably higher than about 100 fmoles/cm^2 of solid support surface.

[0142] The capture nucleotide sequences specific for the determination of a group of organisms are designed in a way as to be able to specifically capture the different sequences belonging to the various groups. These capture nucleotide sequences are called consensus for this group of organisms. The consensus capture nucleotide sequences may contain specific sequences which are longer than the specific capture nucleotide sequences of the different members of the group. These capture nucleotide sequences are consensus sequences, (i.e. the sequences containing at each of its location the base which is the most present in the different sequences of the members of the group when aligned). In another embodiment the consensus capture nucleotide sequence has the length of the amplified sequences.

[0143] The inventors have found unexpected results in that the same identification of several organisms of several groups can be performed at the organisms as well as at the level in the same experimental conditions. Identification of the groups required long capture nucleotide sequences while the specific identification of the organism require small, but specific capture sequences. The inventors found that using the characteristic of the invention, mainly by binding of the specific part of the sequences onto a spacer, it was possible to obtain both results in the same experimental conditions; The invention allows also to use the same stringency conditions, meanly determined by the salt concentration and the temperature and the rate of reaction.

[0144] According to the invention, organisms are identified as such by their specific polymorphism. Single base substitution in a particular location of genome is the characteristic of an individual organism among others of the same species. The method for identification of the polymorphism is part of the invention with direct hybridization of the amplified sequences on the capture nucleotide sequences of the array and detection of the fixed target sequence.

[0145] The detection of the target sequence being bound on capture nucleotide sequences is obtained through the labeling of the capture nucleotide sequence on which the target sequence is bound. A step of capture nucleotide sequences labeling is added after the hybridization step. The extension of the capture nucleotide sequence free end, preferably the 3' end) is performed using detectable nucleotide, preferably a biotin or fluorescent nucleotide, and a polymerization agent, preferably a DNA polymerase and the necessary reagent for making the extension. The target sequence hybridized on the capture nucleotide sequence serves as matrix for the extension; the hybridized target sequences are then removed from the capture nucleotide sequence, rehybridized and extension of the capture nucleotide sequence performed.

[0146] The invention allows identification of the presence of a polymorphism by using an array having at least five different bounded single stranded capture polynucleotide sequence/cm^2 of solid support surface, the determination of a single signal resulting from the binding between the capture sequence and the target sequence, extending at least one polynucleotide primer of the hybrid beyond the 3' terminal nucleotide thereof in the 3' 5' direction using the polynucleotide sequence as a template, said extension is effected in the presence of polymerization agent and nucleotide precursor wherein at least one nucleotide incorporated into the extended primer molecule is a detectably-modified nucleotide; denaturing the duplex to free the target sequence from the polynucleotide capture nucleotide sequence, carry out step one or more times and detecting the presence of a signal associated with the detectable modified nucleotide in the extended capture nucleotide sequence at the reaction zone to effect said determination.

[0147] The process is repeated as needed to obtain a signal detectable on the array. A preferred signal is obtained in colorimetry using the silver precipitation as proposed and detection of the array on colorimetric detector (WO 00/72018). The arrays may be

present in the surface of multiwells and multiwells plate detectors used for the reading of the results.

[0148] In another embodiment, a second labeled nucleotide sequence complementary to the target sequence and adjacent to the capture nucleotide sequence is added on the hybridized amplicons and a ligation performed. If the last base of the capture nucleotide sequence is complementary to the target sequence, then ligation will occur and the spot is labeled. If not ligation will not occur even if the target amplicon is hybridized on the capture nucleotide sequence.

[0149] In a particular embodiment the array bear in separated area several identical capture nucleotide sequences differing only by one nucleotide located at the same place in the capture nucleotide sequence, the last free end is the interrogation base. The array is then able to identify the presence of any of the 4 bases present at a given location of the sequence. Such array is especially useful when detecting polymorphism in homozygote or heterozygote organism or when the polymorphism is not known.

[0150] In the method, kit (device) or apparatus according to the invention, the portion(s) (or part(ies)) of the capture nucleotide sequences complementary to the target sequence is composed of at least two families. The first one comprised between about 5 and about 60 bases, preferably between about 15 and about 40 bases and more preferably between about 20 and about 30 bases. In the second capture family, the binding parts of the capture nucleotide sequence sequences are comprised between about 10 and 1000 bases and preferably between 100 and 600 bases. These bases are preferably assigned as a continuous sequence located at or near the extremity of the capture nucleotide sequence. This sequence is considered as the specific sequence for the detection. In a preferred form of the invention, the sequence located between the specific capture nucleotide sequence and the support surface is a non-specific sequence.

[0151] In another preferred embodiment of the invention, the first family of capture nucleotide sequences detect the members of a group while the second family of capture nucleotide sequences detect the group as such.

[0152] However, both families of capture nucleotide sequences can be polynucleotides.

[0153] All the capture sequences present on the array necessary for capturing the target sequences are polynucleotides and are able to detect both the members of a group and the groups or sub-groups themselves.

[0154] The consensus primers can be chosen in order to amplify different sequences and groups of sequences.

[0155] The same pair of primers amplifies several groups of sequences being different for the different groups of homologous sequences, each one being associated with one or several group of organism.

[0156] The pair of consensus primers may be associated with group identification and/or for species identification on the array.

[0157] A second or third (or even more) primers are added for the amplification step in order to possibly amplify other sequences, related or not to one particular group and useful to be detected in the sample. Virus susceptible to be present in a clinical sample together with bacteria is one of the examples where such extension of the invention is particularly useful like the combination of virus detection of example 17 with bacteria detection of examples 7, 8 or 16.

[0158] Two pairs of (possibly consensus) primers may be used for the amplification, (one for amplification of sequences of the gram-positive and the other one for the gram-negative bacteria, the amplified sequences are specific of each of the gram-positive or the gram-negative bacteria and detected thereafter on the array as specific bacteria species or/and genus and/or family).

[0159] Each of the two primers pair amplifies various sequences specific of one or several families which are then detected as specific species or/and genus, families on the array.

[0160] The same array can also bear capture nucleotides sequences specific for bacterial families or genus.

[0161] In one preferred embodiment of the invention, the detection of the presence of any member of the groups are first detected during the PCR using method like the real time PCR and the amplicons are thereafter used for identification on the array.

[0162] Real time PCR is performed in specific machines which along the PCR cycle detect the appearance of fluorescence in the solution. Increase in fluorescence is

due to the insertion of fluorochromes such as in the double stranded amplicons produced during the PCR cycles.

[0163] Specific fluorescent labeled nucleotide sequences are added to the PCR solution for specific identification of the amplicons. These nucleotide sequences are complementary to the amplified target sequences and their fluorescence emission is limited by the presence at the right position of a scavenger. (Once digested by the polymerase during the copying of the amplicons, the fluorochrome is released in solution where it is detected. Said method is called Fluorescence Resonance Emission Transfert (FRET). The sequence is chosen so as to bind to a consensus region of the detected amplicons or several nucleotide sequences are chosen in consensus regions specific of the groups of sequences or organisms to be detected. (These nucleotide sequences are preferably labeled with different fluorochromes so as to identify the group during the amplification step).

[0164] The fluorescent signal of the amplification solution is registered and if crossing a threshold, the solution is processed for hybridization on capture nucleotide sequences of the array. In a preferred embodiment a solid support bearing the array is added in the amplification chamber and in the hybridization processes. In another preferred embodiment the hybridization is performed on the surface of the same chamber as the PCR. Chambers, preferably closed chambers, can be of any size, format and material as compatible with arrays as already mentioned here above. The chambers may be in polymers such as polycarbonate, polypropylene, or glass such as capillaries. Polyacrylate based surfaces are particularly useful since they are transparent to light and allow covalent binding of capture probes necessary for the arrays. The free end, of the capture nucleotide sequence can be either a 5' or 3' -OH or phosphate group modified in order to avoid elongation. Preferably, the specific sequence portion of the capture nucleotide sequence has a melting temperature smaller than the primers used for the amplification in order to avoid hybridization during the PCR cycles. Also the hybridization may be performed at a given temperature using the heating and control system of the amplification cycler. A control process provides on the amplification cycler to continue or not the detection on the array after the amplification steps.

[0165] The real time PCR may be performed with the primers amplifying the gram-positive or/and the gram-negative PCR and thereafter the families or/and the genes or/and the species identified on the array.

[0166] One embodiment of the invention is to combine in one process the real time PCR together with the hybridization on capture probes for identification of the target molecules or organisms. In a preferred embodiment the process is performed in the same chamber and with the same machine device.

[0167] The present invention also covers the machine and apparatus necessary for performing the various steps of the process mainly for diagnostic and/or quantification of a (micro)organism or component possibly present in a sample among at least two, preferably at least 4 other related (micro)organisms which comprises:

[0168] capture molecules being bound to an insoluble solid support at specific locations according to an array, said capture molecules being able to discriminate between related (micro)organisms or components, said array having a density of at least 4 discrete regions per cm^2 solid support surface

[0169] a detection and/or quantification device of a signal formed at the location of the binding between said target compound with said capture molecule

[0170] possibly reading device of information recorded upon said solid support

[0171] a computer program to recognize the discrete regions bearing the target molecules and their locations

[0172] correlating the presence of the signal at these locations with the detection and/or quantification of the said (micro)organism or component

[0173] in a particular embodiment, this apparatus also performs the genetic amplification of the nucleotide sequences by PCR performed previously or in real time together with the identification of a (micro)organism or its components.

[0174] Detection of other sequences can be advantageously performed on the same array (i.e. by allowing an hybridization with a standard nucleotide sequence used for the quantification, with consensus capture nucleotide sequences for the same or different micro-organisms strains, with a sequence allowing a detection of a possible antibiotic resistance gene by micro-organisms or for positive or negative control of hybridization). Said other capture nucleotide sequences have (possibly) a specific sequence longer than 10 to 60 bases and a total length as high as 600 bases and are also bound upon the insoluble solid support (preferably in the array made with the other bound capture nucleotide sequences related to the invention).

[0175] These characteristics described in details for a specific detection and analysis of nucleotide sequences can be adapted by the person skilled in the art for other components of (micro)organisms such as receptors, antibodies, enzymes, etc.

[0176] The present invention will be described in details in the following non-limiting examples in reference to the enclosed figures and tables.

Brief Description of the Tables

[0177] Table 1 presents identification of 3 gram-positive and 1 gram-negative bacteria at the genus level (horizontally) and at the species level (vertically). These bacteria are detected with the method of the invention on biochips after PCR amplification with consensus primers. The PCR was realized on the gyrase (sub-unit A) sequences.

[0178] The identification of meat animals at the family level (horizontally) and at the genus and species levels (vertically) (3 levels of classification), detected with the method of the invention on biochips after PCR amplification with consensus primers. The PCR was realized on *Cytochrome B* gene sequences.

[0179] Table 3 presents the identification of fishes at the family level (horizontally) and at the genus and species levels (vertically) (3 levels of classification), detected with the method of the invention on biochips after PCR amplification with consensus primers. The PCR was realized on CytochromB gene sequences.

Examples

Example 1: Detection of homologous FemA sequences on array bearing long specific capture nucleotide sequences

[0180] *Production of the capture nucleotide sequences and of the targets*

[0181] The *FemA* genes corresponding to the different *Staphylococci* species were amplified separately by PCR using the following primers:

[0182] *S. aureus 1* : 5' CTTTTGCTGATCGTGATGACAAA 3' (SEQ ID NO: 1)

[0183] *S. aureus 2* : 5' TTTATTTAAAATATCACGCTCTTCG 3' (SEQ ID NO: 2)

[0184] *S. epidermidis 1* : 5' TCGCGGTCCAGTAATAGATTATA 3' (SEQ ID NO: 3)

[0185] *S. epidermidis 2* : 5' TGCATTTCCAGTTATTTCTCCC 3' (SEQ ID NO: 4)

[0186] *S. haemolyticus 1* : 5' ATTGATCATGGTATTGATAGATAC 3' (SEQ ID NO: 5)

[0187] *S. haemolyticus 2* : 5' TTTAATCTTTTTGAGTGTCTTATAC 3' (SEQ ID NO: 6)

[0188] *S. saprophyticus 1* : 5' TAAAATGAAACAACTCGGTTATAAG 3'(SEQ ID NO:7)

[0189] *S. saprophyticus 2* : 5' AAACTATCCATACCATTAAGTACG 3' (SEQ ID NO: 8)

[0190] *S. hominis 1* : 5' CGACCAGATAACAAAAAAGCACAA 3' (SEQ ID NO: 9)

[0191] *S. hominis 2* : 5' GTAATTCGTTACCATGTTCTAA 3' (SEQ ID NO: 10)

[0192] The PCR was performed in a final volume of 50 µl containing: 1.5 mM $MgCl_2$, 10 mM Tris pH 8.4, 50 mM KCl, 0.8 µM of each primer, 50 µM of each dNTP, 50 µM of biotin-16-dUTP), 1.5 U of Taq DNA polymerase Biotools, 7.5% DMSO, 5 ng of plasmid containing *FemA* gene. Samples were first denatured at 94 °C for 3 min. Then 40 cycles of amplification were performed consisting of 30 sec at 94 °C, 30 sec at 60 °C and 30 sec at 72 °C and a final extension step of 10 min at 72 °C. Water controls were used as negative controls of the amplification. The sizes of the amplicons obtained using these primers were 108 bp for *S. saprophyticus*, 139 bp for *S. aureus*, 118 bp for *S. hominis*, 101 bp for *S. epidermidis* and 128 bp for *S. haemolyticus*. The sequences of the capture nucleotide sequences were the same as the corresponding amplicons but they were single strands.

[0193] The biochips also contained positive controls which were CMV amplicons hybridized on their corresponding capture nucleotide sequence and negative controls which were capture nucleotide sequences for a HIV-I sequence on which the CMV could not bind.

Capture nucleotide sequence immobilization

[0194] The protocol described by Schena et al (Proc. Natl Acad. Sci. USA 93, 10614 (1996)) was followed for the grafting of aminated DNA to aldehyde derivatized glass. The aminated capture nucleotide sequences were spotted from solutions at concentrations ranging from 150 to 3000 nM. The capture nucleotide sequences were printed onto the silylated microscopic slides with a home made robotic device (250 µm pins from Genetix (UK) and silylated (aldehyde) microscope slides from Cell associates (Houston, USA)). The spots have 400 µm in diameter and the volume dispensed is about 0.5 nl. Slides were dried at room temperature and stored at 4 °C until used.

Hybridization

[0195] At 65 µl of hybridization solution (AAT, Namur, Belgium) were added 5 µl of amplicons and the solution was loaded on the array framed by an hybridization chamber. For positive controls we added 2 nM biotinylated CMV amplicons of 437 bp to the solution; their corresponding capture nucleotide sequences were spotted on the array. The chamber was closed with a coverslip and slides were denatured at 95 °C for 5 min. The hybridization was carried out at 60° for 2 h. Samples were washed 4 times with a washing buffer.

Colorimetric detection

[0196] The glass samples were incubated 45 min at room temperature with 800 µl of streptavidin labeled with colloidal gold 1000 x diluted in blocking buffer (Maleic buffer 100 mM pH 7.5, NaCl 150 mM, Gloria milk powder 0.1%). After 5 washes with washing buffer, the presence of gold served for catalysis of silver reduction using a staining revelation solution (AAT, Namur, Belgium). The slides were incubated 3 times 10 min with 800 µl of revelation mixture, then rinsed with water, dried and analyzed using a microarray reader. Each slides were then quantified by a specific quantification software.

Fluorescence detection

[0197] The glass samples were incubated 45 min at room temperature with 800 µl of Cyanin 3 or Cyanin 5 labeled streptavidin. After washing, the slides were dried before being stored at room temperature. The detection was performed in the array-scanner GSM 418 (Genetic Microsystem, Woburn, MA, USA) Each slide was then quantified by a specific quantification software.

[0198] The results give a cross-reaction between the species. For example, *epidermidis* amplicons hybridized on its capture nucleotide sequence give a value of 152, but give a value of 144, 9, 13 and 20 respectively for the *S. saprophyticus*, *S. aureus*, *S. haemolyticus* and *S. hominis* capture nucleotide sequences.

Example 2: Detection of homologous FemA sequences on array bearing small specific capture nucleotide sequences

[0199] Protocols for capture nucleotide sequences immobilization and silver staining detection were described in example 1 but the capture nucleotide sequences specific of the 5 *Staphylococcus* species were spotted at concentrations of 600 nM and are the following:

Name	Sequence (5' -> 3')
Capture nucleotide sequence	
ATaur02	ATTTAAAATATCACGCTCTTCGTTTAG (SEQ ID NO: 11)
ATepi02	ATTAAGCACATTTCTTTCATTATTTAG (SEQ ID NO: 12)
AThae02	ATTTAAAGTTTCACGTTCATTTTGTAA (SEQ ID NO: 13)
AThom02	ATTTAATGTCTGACGTTCTGCATGAAG (SEQ ID NO: 14)
ATsap02	ACTTAATACTTCGCGTTCAGCCTTTAA (SEQ ID NO: 15)

[0200] In this case, the targets are fragments of the FemA gene sequence corresponding to the different Staphylococci species which were amplified by a PCR using the following consensus primers:

[0201] APstap03: 5' CCCACTCGCTTATATAGAATTTGA 3' (SEQ ID NO: 16)

[0202] APstap04: 5' CCACTAGCGTACATCAATTTTGA 3' (SEQ ID NO: 17)

[0203] APstap05: 5' GGTTTAATAAAGTCACCAACATATT 3' (SEQ ID NO: 18)

[0204] This PCR was performed in a final volume of 100 µl containing: 3 mM $MgCl_2$, 1 mM Tris pH 8, 1 µM of each primer, 200 µM of dATP, dCTP and dGTP, 150 µM of dTTP, 50 µM of biotin-16-dUTP, 2.5 U of Taq DNA polymerase (Boehringer Mannheim, Allemagne), 1 U of Uracil-DNA-glycosylase heat labile (Boehringer Mannheim, Allemagne), 1 ng of plasmid containing *FemA* gene. Samples were first denatured at 94°C for 5 min. Then 40 cycles of amplification were performed consisting of 1 min at 94°C, 1 min at 50°C and 1 min at 72°C and a final extension step of 10 min at 72°C. Water controls were used as negative controls of the amplification. The sizes of the amplicons obtained using these primers were 489 bp for all species.

[0205] The hybridization solution was prepared as in example 1 and loaded on the slides. Slides were denatured at 98°C for 5 min. Hybridization are carried out at 50°C for 2h. Samples are then washed 4 times with a washing buffer. The values were very low and almost undetectable.

Example 3: Effect of the spacer length on the Sensitivity of detection of homologous FemA sequences on array bearing long capture nucleotide sequences with a small specific sequence

[0206] The experiment was conducted as described in example 2 with the same amplicons but the capture nucleotide sequences used are the following:

Name	Sequence (5' -> 3')
Capture nucleotide sequence	
Ataur02	ATTTAAAATATCACGCTCTTCGTTTAG (SEQ ID NO: 11)
ATepi02	ATTAAGCACATTTCTTTCATTATTTAG (SEQ ID NO: 12)

ATepi03	GAATTCAAAGTTGCTGAGAAATTAAGCACATTTCTTTCATTATTTAG (SEQ ID NO: 19)
ATepi04	GAATTCAAAGTTGCTGAGAATAGTTCAATGGAAGGAAGCGATTAAGCACATTTCTTTCATTATTTAG (SEQ ID NO: 20)
ATepi05	GAATTCAAAGTTGCTGAGAATAGTTCAATGGAAGGAAGCGTCTTCTTAAAATCTAAAGAAATTAAGCACATTTCTTTCATTATTTAG (SEQ ID NO: 21)

[a]The spacer sequences are underlined

[0207] The target amplicons were 489 bp long while the capture nucleotide sequences were 47, 67 or 87 bases single stranded DNA with a specific sequence of 27 bases.

Example 4: Specificity of the detection of FemA sequences from different bacterial species on the same array bearing long capture nucleotide sequences with a small specific sequence

[0208] The experiment was conducted as described in example 2 but the capture nucleotide sequences were spotted at concentrations of 3000 nM and are the following:

Name	Sequence (5' -> 3')
Capture nucleotide sequence	
Ataur27	GAATTCAAAGTTGCTGAGAATAGTTCAATGGAAGGAAGCGATTTAAAATATCACGCTCTTCGTTTAG (SEQ ID NO: 22)
Atepi27	GAATTCAAAGTTGCTGAGAATAGTTCAATGGAAGGAAGCGATTAAGCACATTTCTTTCATTATTTAG (SEQ ID NO: 23)

Athae27	GAATTCAAAGTTGCTGAGAATAGTTCAATGGAAGGAAGC GATTTAAAGTTTCACGTTCATTTTGTAA (SEQ ID NO: 24)
Athom27	GAATTCAAAGTTGCTGAGAATAGTTCAATGGAAGGAAGC GATTTAATGTCTGACGTTCTGCATGAAG (SEQ ID NO: 25)
Atsap27	GAATTCAAAGTTGCTGAGAATAGTTCAATGGAAGGAAGC GACTTAATACTTCGCGTTCAGCCTTTAA (SEQ ID NO: 26)

[a]The spacer sequence is underlined. The specific sequences were of 27 bases

[0209] The targets are fragments of the *FemA* gene sequence corresponding to the different Staphylococci species which were amplified by PCR using the following consensus primers:

[0210] APcons3-1: 5' TAAYAAARTCACCAACATAYTC 3' (SEQ ID NO: 27)

[0211] APcons3-2: 5' TYMGNTCATTTATGGAAGATAC 3' (SEQ ID NO: 28)

[0212] A consensus sequence is present on the biochips which detects all the tested *Staphylococcus* species. All target sequences were amplified by PCR with the same pair of primers.

[0213] The size of the amplicons obtained using these primers were 587 bp for all species. The consensus sequence capture nucleotide sequence was a 489 base long single stranded DNA complementary to the amplicons of *S. hominis* as amplified in example 2. The detection was made in fluorescence. Homology between the consensus capture nucleotide sequence and the sequences of the *FemA* from the 15 S. species were between 66 and 85%. All the sequences hybridized on this consensus capture nucleotide sequence.

Example 5: effect of the length of the specific sequence of the capture nucleotide sequence on the discrimination between homologous sequences

[0214] The experiment was conducted as described in example 4 but at a temperature of 43°C and the capture nucleotide sequences used are presented in the table here joined. The numbers after the names indicate the length of the specific sequences.

[0215] The *FemA* amplicons of *S. anaerobius* (a subspecies of *S. aureus*) were hybridized on an array bearing capture nucleotide sequences of 67 single stranded bases with either 15, 27 and 40 bases specific for the *S. aureus*, *anaerobius* and *epidermidis* at their extremities. The difference between the capture nucleotide sequences of *anaerobius* and *aureus* was only one base in the 15 base capture nucleotide sequence and 2 in the 27 and the 40 bases.

[0216] The amplicons of the *FemA* from the three *Staphylococcus* species were hybridized on the array.

Name	Sequence (5' -> 3')
Capture nucleotide sequence	
Ataur15	GAATTCAAAGTTGCTGAGAATAGTTCAATGGAAGGAAG CGTCTTCTTAAAATGCTCTTCGTTTAGTT (SEQ ID NO: 29)
Ataur27	GAATTCAAAGTTGCTGAGAATAGTTCAATGGAAGGAAG CGATTTAAAATATCGCTCTTCGTTTAG (SEQ ID NO: 22)
Ataur40	GAATTCAAAGTTGCTGAGAATAGTTCAAATCTTTATTTA AAATATCACGCTCTTCGTTTAGTTCTTT (SEQ ID NO: 30)
Atana15	GAATTCAAAGTTGCTGAGAATAGTTCAATGGAAGGAAG CGTCTTCTTAAAATGCTCTTCATTTAGTT (SEQ ID NO: 31)
Atana27	GAATTCAAAGTTGCTGAGAATAGTTCAATGGAAGGAAG CGGTTTAAAATATCACGCTCTTCATTTAG (SEQ ID NO: 32)

Atana40	GAATTCAAAGTTGCTGAGAATAGTTCAAATCTTTGTTTA AAATATCACGCTCTTCATTTAGTTCTTT (SEQ ID NO: 33)
Atepi15	GAATTCAAAGTTGCTGAGAATAGTTCAATGGAAGGAAG CGTCTTCTTAAAATTTTCATTATTTAGTT (SEQ ID NO: 34)
Atepi27	GAATTCAAAGTTGCTGAGAATAGTTCAATGGAAGGAAG CGATTAAGCACATTTCTTTCATTATTTAG (SEQ ID NO: 23)
Atepi40	GAATTCAAAGTTGCTGAGAATAGTTCAAATCTTTATTAA GCACATTTCTTTCATTATTTAGTTCCTC (SEQ ID NO: 35)

Example 6: Sensitivity of the detection of FemA sequences of Staphylococcus aureus on arrays bearing specific sequence as proposed by this invention and the consensus sequence

[0217] The experiment was conducted as described in example 4 with the capture nucleotide sequences spotted at concentrations of 3000 nM. The bacterial *FemA* sequences were serially diluted before the PCR and being incubated with the arrays.

Example 7: Detection of 16 homologous FemA sequences on array

The consensus primers and the amplicons were the same as described in the example 4 but the capture probes were chosen for the identification of 15 Staphylococcus species. The experiment is conducted as in example 4. The capture nucleotide sequences contain a spacer fixed on the support by its 5' end and of the following sequence 5' GAATTCAAAGTTGCTGAGAATAGTTCAATGGAAGGAAGCG 3' (SEQ ID NO: 36) followed by the following specific sequences for the various femA from the different Staphylococcus.

[0218] *S. aureus*: ATTTAAAATATCACGCTCTTCGTTTAG (SEQ ID NO: 37)

[0219] *S. epidermidis*: ATTAAGCACATTTCTTTCATTATTTAG (SEQ ID NO: 38)

[0220] *S. haemolyticus*: ATTTAAAGTTTCACGTTCATTTTGTAA (SEQ ID NO: 39)

[0221] *S. hominis*: ATTTAATGTCTGACGTTCTGCATGAAG (SEQ ID NO: 40)

[0222] *S. saprophyticus*: ACTTAATACTTCGCGTTCAGCCTTTAA (SEQ ID NO: 41)

[0223] *S. capitis*: ATTAAGAACATCTCTTTCATTATTAAG (SEQ ID NO: 42)

[0224] *S. caseolyticus*: ATAAAGACATTCGAGACGAAGGCT (SEQ ID NO: 43)

[0225] *S. cohnii*: ACTTAACACTTCACGCTCTGACTTGAG (SEQ ID NO: 44)

[0226] *S. gallinarum*: ACTTAAAACTTCACGTTCAGCAGTAAG (SEQ ID NO: 45)

[0227] *S. intermedius*: GTGGAAATCTTGCTCTTCAGATTTCAG (SEQ ID NO: 46)

[0228] *S. lugdunensis*: TTCTAAAGTTTGTCGTTCATTCGTTAG (SEQ ID NO: 47)

[0229] *S. schleiferi*: TTTAAAGTCTTGCGCTTCAGTGTTGAG (SEQ ID NO: 48)

[0230] *S. sciuri*: GTTGTATTGTTCATGTTCTTTTTCTAA (SEQ ID NO: 49)

[0231] *S. simulans:* TTCTAAATTCTTTTGTTCAGCGTTCAA (SEQ ID NO: 50)

[0232] *S. warneri:* AGTTAAGGTTTCTTTTTCATTATTGAG (SEQ ID NO: 51)

[0233] *S. xylosus*: GCTTAACACCTCACGTTGAGCTTGCAA (SEQ ID NO: 52)

Example 8: Detection of 13 homologous p34 Sequences and identification of 13 Mycobacteria species

[0234] The *P34* genes present in all *Mycobacteria* were all amplified with the following consensus primers:

Sense

[0235] MycU4 5' CATGCAGTGAATTAGAACGT 3' (SEQ ID NO: 53) located at the position 496-515 of the gene, Tm = 56°C

Antisense

[0236] APmcon02 5' GTA**S**GTCAT**RRSTY**CTCC 3' (SEQ ID NO: 54) located at the position 733-750 of the gene, Tm = 52-58°C

[0237] S = C or G

[0238] R = A or G

[0239] Y = T or C

[0240] The size of amplified products ranges from 123 to 258 bp.

[0241] The following capture nucleotide sequences were chosen for the specific capture of the Mycobacteria sequences:

Capture nucleotide sequences

[0242] M. avium : 5' CGGTCGTCTCCGAAGCCCGCG 3' (21 nt) (SEQ ID NO: 55)

[0243] M. gastrii 1 : 5' GATCGGCAGCGGTGCCGGGG 3' (20 nt) (SEQ ID NO: 56)

[0244] M. gastrii 3 : 5' GTATCGCGGGCGGCAAGGT 3' (19 nt) (SEQ ID NO: 57)

[0245] M. gastrii 5 : 5' TCTGCCGATCGGCAGCGGTGCCGG 3' (24nt) (SEQ ID NO: 58)

[0246] M. gastrii 7 : 5' GCCGGGGCCGGTATTCGCGGGCGG 3' (24nt) (SEQ ID NO: 59)

[0247] M. gordonae : 5' GACGGGCACTAGTTGTCAGAGG 3' (22 nt) (SEQ ID NO: 60)

[0248] M. intracellulare 1: 5' GGGCCGCCGGGGGCCTCGCCG 3' (21 nt) (SEQ ID NO: 61)

[0249] M. intracellulare 3 : 5' GCCTCGCCGCCCAAGACAGTG 3' (21 nt) (SEQ ID NO: 62)

[0250] M. leprae: 5' GATTTCGGCGTCCATCGGTGGT 3' (22 nt) (SEQ ID NO: 63)

[0251] M. kansasi 1 : 5' GATCGTCGGCAGTGGTGACGG 3' (21 nt) (SEQ ID NO: 64)

[0252] M. kansasi 3 : 5' TCGTCGGCAGTGGTGAC 3' (17 nt) (SEQ ID NO: 65)

[0253] M. kansasi 5 : 5' ATCCGCCGATCGTCGGCAGTGGTGACG 3' (27 nt)

(SEQ ID NO: 66)

[0254] M. malmoense : 5' GACCCACAACACTGGTCGGCG 3' (21 nt) (SEQ ID NO: 67)

[0255] M. marinum : 5' CGGAGGTGATGGCGCTGGTCG 3' (21 nt) (SEQ ID NO: 68)

[0256] M. scrofulaceum : 5' CGGCGGCACGGATCGGCGTC (20 nt) (SEQ ID NO: 69)

[0257] M. simiae: 5' ATCGCTCCTGGTCGCGCCTA 3' (20 nt) (SEQ ID NO: 70)

[0258] M. szulgai : 5' CCCGGCGCGACCAGCAGAACG 3' (21 nt) (SEQ ID NO: 71)

[0259] M. tuberculosis: 5' GCCGTCCAGTCGTTAATGTCGC 3' (22 nt) (SEQ ID NO: 72)

[0260] M. xenopi: 5' CGGTAGAAGCTGCGATGACACG 3' (22 nt) (SEQ ID NO: 73)

[0261] Each of the sequences above comprises a spacer at its 5' end. Spacer sequence: 5' GAATTCAAAGTTGCTGAGAATAGTTCAATGGAAGGAAGCGTCTTC 3' (SEQ ID NO: 74). Capture nucleotide sequences were aminated at their 5' end.

Example 9: Detection of MAGE genes

[0262] MAGE genes were all amplified with the following consensus primers:

Sense

- DPSCONS2 5' GGGCTCCAGCAGCCAAGAAGAGGA 3' (SEQ ID NO: 75), located at the 398-421 position of the gene

[0263] Tm = 78°C

[0264] Other amplicons were added as sense primer in order to increase the efficiency of the PCR for some MAGEs:

- DPSMAGE1 5' GGGTTCCAGCAGCCGTGAAGAGGA 3' (SEQ ID NO: 76)

[0265] Tm = 78°C

- DPSMAG8 5' GGGTTCCAGCAGCAATGAAGAGGA 3' (SEQ ID NO: 77) Tm = 74°C

- DPSMAG12 5' GGGCTCCAGCAACGAAGAACAGGA 3' (SEQ ID NO: 78)

[0266] Tm = 76°C

Antisense

- DPASCONB4 5' CGGTACTCCAGGTAGTTTTCCTGC 3' (SEQ ID NO: 79), located at the position 913-936 of the gene, Tm = 74°C

[0267] The size of the amplified products are around 530 bp.

[0268] The following capture nucleotide sequences of 27 nucleotides were chosen for the specific capture of the MAGE sequences:

Capture nucleotide sequences

[0269] Mage 1 DTAS01 5' ACAAGGACTCCAGGATACAAGAGGTGC 3' (SEQ ID NO: 80)

[0270] Mage 2 DTAS02 5' ACTCGGACTCCAGGTCGGGAAACATTC 3' (SEQ ID NO: 81)

[0271] Mage 3 DTS0306 5' AAGACAGTATCTTGGGGGATCCCAAGA 3' (SEQ ID NO: 82)

[0272] Mage 4 DTAS04 5' TCGGAACAAGGACTCTGCGTCAGGCGA 3' (SEQ ID NO: 83)

[0273] Mage 5 DTAS05 5' GCTCGGAACACAGACTCTGGGTCAGGG 3' (SEQ ID NO: 84)

[0274] Mage 6 DTS06 5' CAAGACAGGCTTCCTGATAATCATCCT 3' (SEQ ID NO: 85)

[0275] Mage 7 DTAS07 5' AGGACGCCAGGTGAGCGGGGTGTGTCT 3' (SEQ ID NO: 86)

[0276] Mage 8 DTAS08 5' GGGACTCCAGGTGAGCTGGGTCCGGGG 3' (SEQ ID NO: 87)

[0277] Mage 9 DTAS09 5' TGAACTCCAGCTGAGCTGGGTCGACCG 3' (SEQ ID NO: 88)

[0278] Mage 10 DTAS10 5' TGGGTAAAGACTCACTGTCTGGCAGGA 3' (SEQ ID NO: 89)

[0279] Mage 11 DTAS11 5' GAAAAGGACTCAGGGTCTATCAGGTCA 3' (SEQ ID NO: 90)

[0280] Mage 12 DTAS12 5' GTGCTACTTGGAAGCTCGTCTCCAGGT 3' (SEQ ID NO: 91)

[0281] Each of the sequences above comprises a spacer aminated at its 5' end in order to be covalently linked to the glass. Spacer sequence 5' GAATTCAAAGTTGCTGAGAATAGTTCAATGGAAGGAAGCG 3' (SEQ ID NO: 36)

[0282] They were spotted on aldehyde bearing glasses and used for the detection of the MAGEs amplified by the consensus primers given here above. The results showed a non equivocal identification of the MAGEs present in the tumors compared to identification using 12 specific PCR, one for each MAGE sequences.

Example 10: Identification of G-protein dopamine receptors subtypes in rat

[0283] Dopamine Receptor coupled to the G-protein were all amplified with the following consensus primers:

Sense

- CONSENSUS2-3-4

[0284] 5' TGCAGACMACCACCAACTACTT 3' (SEQ ID NO: 92) located at the position 221-242 of the gene, Tm = 66°C

[0285] M = A or C

- CONSENSUS1-5

[0286] 5' TGMGGKCCAAGATGACCAACWT 3' (SEQ ID NO: 93) (22 nt) located at the position 221-240 of the gene, Tm = 66°C

[0287] M = A or C

[0288] K = G or T

[0289] W = A or T

Antisense

[0290] 5' TCATGRCRCASAGGTTCAGGAT 3' (SEQ ID NO: 94) located at the position 395-416 of the gene, Tm = 64-68°C

[0291] R = A or G

[0292] S = C or G

[0293] The size of the amplified product is 196 bp.

[0294] The following capture nucleotide sequences of 27 nucleotides were chosen for the specific capture of the dopamine receptor sequences:

Capture nucleotide sequences

[0295] DRD1 5' CTGGCTTTTGGCCTTTGGGTCCCTTTT 3' (SEQ ID NO: 95)

[0296] DRD2 5' TGATTGGAAATTCAGCAGGATTCACTG 3' (SEQ ID NO: 96)

[0297] DRD3 5' GAGTCTGGAATTTCAGCCGCATTTGCT 3' (SEQ ID NO: 97)

[0298] DRD4 5' CGTCTGGCTGCTGAGCCCCCGCCTCTG 3' (SEQ ID NO: 98)

[0299] DRD5 5' CTGGGTACTGGCCCTTTGGGACATTCT 3' (SEQ ID NO: 99)

[0300] Each of the sequences above comprised an aminated spacer at its 5' end. Spacer sequence 5' GAATTCAAAGTTGCTGAGAATAGTTCAATGGAAGGAAGCG (SEQ ID NO: 36).

Example 11: Identification of G-protein histamine receptors subtypes in rat

[0301] Histamine Receptor coupled to the G-protein were all amplified with the following primers:

Sense

- H1sense

[0302] 5' CTCCGTCCAGCAACCCCT 3' (SEQ ID NO: 100) (18 nt) located at the Position 381-398 of the gene, Tm = 60°C

- H2sense

[0303] 5' CTGTGCTGGTCACCCCAGT 3' (SEQ ID NO: 101) (19 nt) located at the Position 380-398 of the gene, Tm = 62°C

- H3sense

[0304] 5' ACTCATCAGCTATGACCGATT 3' (SEQ ID NO: 102) (21 nt) located at the Position

[0305] 378-398 of the gene, Tm = 60°C

Antisense

- H1antisense

[0306] 5' ACCTTCCTTGGTATCGTCTG 3' (SEQ ID NO: 103) (20 nt) located at the Position 722-741 of the gene, Tm = 60°C

- H2antisense

[0307] 5' GAAACCAGCAGATGATGAACG 3' (SEQ ID NO: 104) (21 nt) located at the Position 722-742 of the gene, Tm = 62°C

- H3antisense

[0308] 5' GCATCTGGTGGGGGTTCTG 3' (SEQ ID NO: 105) (19 nt) located at the Position 722-740 of the gene, Tm = 62°C

[0309] Size of the amplified product ranged from 359 to 364 bp.

[0310] The following capture nucleotide sequences were chosen for the specific capture of the histamine receptor sequences:

Capture nucleotide sequences

[0311] H1 5' CCCCAGGATGGTAGCGGA 3' (18 nt) (SEQ ID NO: 106)

[0312] H2 5' AGGATAGGGTGATAGAAATAAC 3' (22 nt) (SEQ ID NO: 107)

[0313] H3 5' TCTCGTGTCCCCCTGCTG 3' (18 nt) (SEQ ID NO: 108)

[0314] Each of the sequences above comprised a spacer at its 5' end.

[0315] Spacer sequence 5' GAATTCAAAGTTGCTGAGAATAGTTCAATGGAAGGAAGCG 3' (SEQ ID NO: 36). Capture nucleotide sequences were aminated at their 5' end.

Example 12: Identification of G-protein serotonin receptors subtypes in rat

[0316] Serotonin Receptor coupled to the G-protein were all amplified with the following primers:

Sense

- Consensus for the subtypes 1A, 1B, 1C, 1D, 1E, 2A, 2B, 2C, 4, 6, 7

[0317] 5'ATC**H**TGCACCT**S**TG**B**G**B**CAT 3' (SEQ ID NO: 109) Tm = 58-64°C (20 nt)

[0318] H = C or A or T

[0319] S = C or G

[0320] B = C or T or G

[0321] 1A ATC**C**TGCACCT**G**TG**C**G**C**CAT (0 mismatch) position 370-389 (SEQ ID NO: 110)

[0322] 1B ATC**A**TGCA<u>T</u>CT**C**TG**T**G**T**CAT (1 mismatch) position 397-416 (SEQ ID NO: 111)

[0323] 1C ATC**A**TGCACCT**C**TG**C**G**C**CAT (0 mismatch) position 427-446 (SEQ ID NO: 112)

[0324] 1D ATC**C**TGCA<u>T</u>CT**C**TG**T**G**T**CAT (1 mismatch) position 367-386 (SEQ ID NO: 113)

[0325] 1E ATC**T**TGCACCT**G**T<u>C</u>**G**G**C**<u>T</u>AT (2 mismatches) position 331-350 (SEQ ID NO: 114)

[0326] 2A ATC**A**TGCACCT**C**TG**C**G**C**CAT (0 mismatch) position 487-506 (SEQ ID NO: 115)

[0327] 2B ATC**A**TGCA<u>T</u>CT**C**TG**T**G**C**CAT (1 mismatch) position 424-443 (SEQ ID NO: 116)

[0328] 2C ATC**A**TGCACCT**C**TG**C**G**C**CAT (0 mismatch) position 24-43 (SEQ ID NO: 117)

[0329] 4 AT<u>T</u>**T**T<u>T</u>CACCT**C**TG**C**<u>T</u>**G**CAT (3 mismatches) (SEQ ID NO: 118)

[0330] 6 ATC**C**T<u>CA</u>ACCT**C**TG**C**<u>T</u>**T**CAT (3 mismatches) (SEQ ID NO: 119)

[0331] 7 ATC**A**TG<u>AC</u>CCT**G**TG**C**G**T**<u>G</u>AT (3 mismatches) (SEQ ID NO: 120)

- Consensus 4, 6

[0332] 5' ATC**Y**T**Y**CACCTCTGC**YK**CAT 3' (SEQ ID NO: 121) Tm = 52-64°C (20 nt)

[0333] K = G or T

[0334] Y = T or C

[0335] 4 ATTTTTCACCTCTGCTGCAT (SEQ ID NO: 122) (1 mismatch) position 322-341

[0336] 6 ATCCTCAACCTCTGCCTCAT (SEQ ID NO: 123) (1 mismatch) position 340-359

- Consensus 5A, 5B

[0337] 5' ATCTGGAA**Y**GTG**R**CAGCCAT 3' (SEQ ID NO: 124) Tm = 58-62°C (20 nt)

[0338] Y = T or C

[0339] R = A or G

[0340] 5A ATCTGGAA**T**GTG**A**CAGCAAT (SEQ ID NO: 125) (1 mismatch) position 385-404

[0341] 5B ATCTGGAA**C**GTG**G**C**G**GCCAT (SEQ ID NO: 126) (1 mismatch) position 424-443

- Specific 7

[0342] 5' ATCATGACCCTGTGCGTGAT 3' (SEQ ID NO: 127) Tm = 56°C (18 nt) position 517-536

- Specific 3B

[0343] 5' CTTCCGGAACGATTAGAAA 3' (SEQ ID NO: 128) Tm = 54°C (19 nt) position 404-422

Antisense

- Consensus for the subtypes 1A, 1B, 1C, 1D, 1E, 2A, 2B, 2C, 4, 7 Tm = 48-58 °C

[0344] 5' TTGG**H**N**G**C**Y**TTC**Y**G**B**TC 3' (SEQ ID NO: 129)

[0345] Y = T or C

H = A or T or C

[0346] N = A or C or G or T

[0347] B = C or T or G

[0348] 1A TTCACCGTCTTCCTTTC (4 mismatches) (SEQ ID NO: 130)

[0349] 1B TTGGTGGCTTTGCGCTC (1 mismatch) position 913-929 (SEQ ID NO: 131)

[0350] 1C TTGGAAGCTTTCTTTTC (1 mismatch) position 922-938 (SEQ ID NO: 132)

[0351] 1D TTAGTGGCTTTCCTTTC (2 mismatches) position 877-893 (SEQ ID NO: 133)

[0352] 1E GTGGCTGCTTTGCGTTC (2 mismatches) position 862-878 (SEQ ID NO: 134)

[0353] 2A TTGCACGCCTTTTGCTC (2 mismatches) position 952-968 (SEQ ID NO: 135)

[0354] 2B TTTGAGGCTCTCTGTTC (2 mismatches) position 952-968 (SEQ ID NO: 136)

[0355] 2C TTGGAAGCTTTCTTTTC (1 mismatch) position 424-440 (SEQ ID NO: 137)

[0356] 4 TTGGCTGCTTTCCGGTC (2 mismatches) (SEQ ID NO: 138)

[0357] 7 GTGGCTGCTTTCTGTTC (1 mismatch) position 973-989 (SEQ ID NO: 139)

- Specific 1A

[0358] 5' TTCACCGTCTTCCTTTC 3' (SEQ ID NO: 140) Tm = 50°C (17 nt) position 1018-1034

- Specific 4

[0359] 5' TCTTGGCTGCTTTGGTC 3' (SEQ ID NO: 141) Tm = 52°C (17 nt) position 762-778

- Specific 6

[0360] 5' ATAAAGAGCGGGTAGATG 3' (SEQ ID NO: 142) Tm = 52°C (18 nt) position 945-963

- Consensus 5A, 5B

[0361] 5' CCTTCTGCTCCCTCCA 3' (SEQ ID NO: 143) Tm = 52°C (16 nt)

[0362] 5A CCTTCTGTTCCCTCCA (1 mismatch) position 823-840 (SEQ ID NO: 144)

[0363] 5B CCTTCTGCTCCCGCCA (1 mismatch) position 862-879 (SEQ ID NO: 145)

- Specific 3B

[0364] 5' ACCGGGGACTCTGTGT 3' (SEQ ID NO: 146) Tm = 52°C (16 nt) position 1072-1089

[0365] The following capture nucleotide sequences were chosen for the specific capture of the serotonin receptor subtypes sequences:

Capture nucleotide sequences

[0366] HTR1C 5' CTATGCTCAATAGGATTACGT 3' (21 nt) (SEQ ID NO: 147)

[0367] HTR2A 5' GTGGTGAATGGGGTTCTGG 3' (19 nt) (SEQ ID NO: 148)

[0368] HTR2B 5' TGGCCTGAATTGGCTTTTTGA 3' (21 nt) (SEQ ID NO: 149)

[0369] HTR2C/1C 5' TTATTCACGAACACTTTGCTTT 3' (22 nt) (SEQ ID NO: 150)

[0370] HTR1B 5' AATAGTCCACCGCATCAGTG 3' (20 nt) (SEQ ID NO: 151)

[0371] HTR1D 5' GTACTCCAGGGCATCGGTG 3' (19 nt) (SEQ ID NO: 152)

[0372] HTR1A 5' CATAGTCTATAGGGTCGGTG 3' (20 nt) (SEQ ID NO: 153)

[0373] HTR1E 5' ATACTCGACTGCGTCTGTGA 3' (20 nt) (SEQ ID NO: 154)

[0374] HTR7 5' GTACGTGAGGGGTCTCGTG 3' (19 nt) (SEQ ID NO: 155)

[0375] HTR5A 5' GGCGCGTTATTGACCAGTA 3' (19 nt) (SEQ ID NO: 156)

[0376] HTR5B 5' GGCGCGTGATAGTCCAGT 3' (18 nt) (SEQ ID NO: 157)

[0377] HTR3B 5' GATATCAAAGGGGAAAGCGTA 3' (21 nt) (SEQ ID NO: 158)

[0378] HTR4 5' AAACCAAAGGTTGACAGCAG 3' (20 nt) (SEQ ID NO: 159)

[0379] HTR6 5' GTAGCGCAGCGGCGAGAG 3' (18 nt) (SEQ ID NO: 160)

[0380] Each of the sequences above comprises a spacer at its 5' end

[0381] Spacer sequence 5' GAATTCAAAGTTGCTGAGAATAGTTCAATGGAAGGAAGCG 3' (SEQ ID NO: 36). Capture nucleotide sequences were aminated at their 5' end.

Example 13 : Identification of the HLA-A subtypes

[0382] The HLA-A subtypes were amplified with the following consensus primers:

Sense

[0383] IPSCONA 5' GACAGCGACGCCGCGAGCCA 3' (SEQ ID NO: 161) located at the position 181-200 of the gene, Tm = 70°C

Antisense

[0384] IPASCONA 5 CGTGTCCTGGGTCTGGTCCTCC 3' (SEQ ID NO: 162) located at the position 735-754 of the gene, Tm = 74°C

[0385] The size of the amplified product was 574 bp.

[0386] The following capture nucleotide sequences of 27 nucleotides were chosen for the specific capture of the HLA-A sequences:

Capture nucleotide sequences

[0387] HLA-A1 ITSA01 5' GGAGGGCCGGTGCGTGGACGGGCTCCG 3' (SEQ ID NO: 163)

[0388] HLA-A2 ITASA02 5' TCTCCCCGTCCCAATACTCCGGACCCT 3' (SEQ ID NO: 164)

[0389] HLA-A3 ITASA03A 5' CTGGGCCTTCACATTCCGTGTCTCCTG 3' (SEQ ID NO: 165)

ITSA03B 5' AGCGCAAGTGGGAGGCGGCCCATGAGG 3' (SEQ ID NO: 166)

[0390] HLA-A11 ITSA11A 5' GCCCATGCGGCGGAGCAGCAGAGAGCC 3' (SEQ ID NO: 167)

ITSA11B 5' CCTGGAGGGCCGGTGCGTGGAGTGGCT 3' (SEQ ID NO: 168)

[0391] HLA-A23 ITSA23A 5' GCCCGTGTGGCGGAGCAGTTGAGAGCC 3' (SEQ ID NO: 169)

ITASA23B 5' CCTTCACTTTCCCTGTCTCCTCGTCCC 3' (SEQ ID NO: 170)

[0392] HLA-A24 ITSA24A 5' GCCCATGTGGCGGAGCAGCAGAGAGCC 3' (SEQ ID NO: 171)

ITASA24B 5' TAGCGGAGCGCGATCCGCAGGTTCTCT 3' (SEQ ID NO:172)

[0393] HLA-A25 ITASA25A 5' TAGCGGAGCGCGATCCGCAGGCTCTCT 3' (SEQ ID NO: 173)

ITASA25B 5' TCACATTCCGTGTGTTCCGGTCCCAAT 3' (SEQ ID NO: 174)

[0394] HLA-A26 ITASA26 5' GGGTCCCCAGGTTCGCTCGGTCAGTCT 3' (SEQ ID NO: 175)

[0395] HLA-A29 ITASA29 5' TCACATTCCGTGTCTGCAGGTCCCAAT 3' (SEQ ID NO: 176)

[0396] HLA-A30 ITASA30 5'CGTAGGCGTGCTGTTCATACCCGCGGA 3' (SEQ ID NO: 177)

[0397] HLA-A31 ITASA31 5' CCCAATACTCAGGCCTCTCCTGCTCTA 3' (SEQ ID NO: 178)

[0398] HLA-A33 ITSA33 5' CGCACGGACCCCCCCAGGACGCATATG 3' (SEQ ID NO: 179)

[0399] HLA-A68 ITSA68A 5' GGCGGCCCATGTGGCGGAGCAGTGGAG 3' (SEQ ID NO: 180)

[0400] ITASA68B 5' GTCGTAGGCGTCCTGCCGGTACCCGCG 3' (SEQ ID NO: 181)

[0401] HLA-A69 ITASA69 5' ATCCTCTGGACGGTGTGAGAACCGGCC 3' (SEQ ID NO: 182)

[0402] Each of the sequences above comprised an aminated spacer at its 5' end. Spacer sequence 5' GAATTCAAAGTTGCTGAGAATAGTTCAATGGAAGGAAGCG 3' (SEQ ID NO: 36)

Example 14: Identification of Cytochrome P450 3a forms

[0403] The Cytochrome P450 forms were amplified with the following consensus primers :

Sense

- Consensus

[0404] 5' GCCAGAGCCTGAGGA 3' (SEQ ID NO: 183) located at the position 1297-1311 of the 3a3 gene, Tm = 50°C

Antisense

- Consensus a3, a23, a1, a2

[0405] 5' TCAAAAGAAATTAACAGAGA 3' (SEQ ID NO: 184) located at the position 1839-1858 of the 3a3 gene, Tm = 50°C

- Specific a9

[0406] 5' ACAATGAAGGTAACATAGG 3' (SEQ ID NO: 185) located at the position 2015-2033 of the 3a9 gene Tm = 52°C

- Specific a18

[0407] 5' ACTGATGGAACTAACTGG 3' (SEQ ID NO: 186) located at the position 1830-1846 of the 3a18 gene Tm = 52°C

[0408] The length of the PCR product was around 560 bp.

[0409] The following capture nucleotide sequences were chosen for the specific capture of the cytochrome P-450 3a sequences:

Capture nucleotide sequence

[0410] *3a1* 5' TGTTTTGATTCGGTACATCTTTG 3' (23 nt) (SEQ ID NO: 187)

[0411] *3a3* 5' TTGATTTGGTACATCTTTGCT 3' (21 nt) (SEQ ID NO: 188)

[0412] 3A9 5' ACTCCTGGGGGTTTTGGGTG 3' (20 nt) (SEQ ID NO: 189)

[0413] 3A18 5' ATTACTGAGTATTCAGAAATTCAC 3' (24 nt) (SEQ ID NO: 190)

[0414] 3A2 5' GGTTAAAGATTTGGTACATTTATGG 3' (25 nt) (SEQ ID NO: 191)

[0415] Each of the sequences above comprised a spacer at its 5' end

[0416] Spacer sequence 5' GAATTCAAAGTTGCTGAGAATAGTTCAATGGAAGGAAGCG 3' (SEQ ID NO: 36). Capture nucleotide sequences were aminated at their 5' end.

Each of the sequences above comprises a spacer at its 5' end

[0417] Spacer sequence 5' GAATTCAAAGTTGCTGAGAATAGTTCAATGGAAGGAAGCG (SEQ ID NO: 36).

Example 15 : Identification of GMO on biochips

[0418] The following primers were chosen for the amplification step of the GMO.

[0419] Consensus primers to detect GMO on biochips:

Forward	Reverse
OPP35S1 (P-35S) 5'CGTCTTCAAAGCAAGTGGATTG3' (SEQ ID NO: 192)	OPT352 (T-35S) 5'GAAACCCTAATTCCCTTATCAGGG3' (SEQ ID NO: 193)
OPTE91 (T-E9) 5'TCATGGATTTGTAGTTGAGTATGAA3' (SEQ ID NO: 194)	OPTnos2 (T-nos) 5'ATCTTAAGAAACTTTATTGCCAAATG T3' (SEQ ID NO: 195)
OPEPS3 (EPSPS) 5'GCTGTAGTTGTTGGCTGTGGT3' (SEQ ID NO: 196)	OPTE92 (T-E9) 5'CTGATGCATTGAACTTGACGA3' (SEQ ID NO: 197)

OPLB1 (octopine Left Border) 5'ATCAGCAATGAGTATGATGGTCAAT3' (SEQ ID NO: 198)	OPEPS4 (EPSPS) 5'GCGACATCAGGCATCTTGTT3' (SEQ ID NO: 199)
OPLB3 (nopaline Left Border) 5'ACAAATTGACGCTTAGACAACT3' (SEQ ID NO: 200)	OPRB2 (octopine Right Border) 5'TGCCAGTCAGCATCATCACAC3' (SEQ ID NO: 201)
	OPRB4 (nopaline Right Border) 5'TAAGGGAGTCACGTTATGACC3' (SEQ ID NO: 202)

[0420] These primers allowed the amplification of the following genes:

[0421] 1) CTP1, CTP2, CP4EPSPS, S CryIAb and hsp 70 Int. in Mon 809 (corn, Monsanto)

[0422] 2) hsp 70 Int. and S CryIAb in Mon 810 (corn, Monsanto)

[0423] 3) S CryIAb and S Pat in Bt 11 (corn, Novartis)

[0424] 4) CTP4 and EPSPS in GTS40-3-2 (soybean, Monsanto)

[0425] The capture nucleotide sequences were chosen in these sequences to allow discrimination. Each of the sequences above comprised a spacer at its 5' end

[0426] Spacer sequence 5' GAATTCAAAGTTGCTGAGAATAGTTCAATGGAAGGAAGCG (SEQ ID NO: 36).

[0427] The following sequences were chosen as specific capture probes of the GMO:

[0428] OT1 pat (T25, Bt11) TGGTGGATGGCATGATGTTGGTTTTTGGCA (SEQ ID NO: 203)

[0429] OT2 CryIAb (Bt11) GCACGAAGCTCTGCAATCGCACAAACCCGT (SEQ ID NO: 204)

[0430] OT3 P-PCK (Bt176) TGGGGGTAGCTGTAGTCGGACTCGGACTGG (SEQ ID NO: 205)

[0431] OT4 CP4EPSPS/Tnos AGCCCCTAGCTAGGGGGTGGCCAGGAAGTA (SEQ ID NO: 206)

Example 16: Detection of gyrase (sub-unit A) sequences on array bearing genus and species specific capture nucleotide sequences Example of bacterial detection

Amplification of the sequences

[0432] The amplified target sequences are fragments of the gyrase gene (sub-unit A) sequences corresponding to the different genus and species (table 1) which were amplified by a PCR using the following consensus primers :

[0433] Pgyr1: 5' GANGTNATSGGTAAATAYCA 3' (SEQ ID NO: 207)

[0434] Pgyr2: 5' CGNRYYTCVGTRTAACG 3' (SEQ ID NO: 208)

[0435] The PCR was performed in a final volume of 100 µl containing: 3 mM $MgCl_2$, 1 mM Tris pH 8, 1 µM of each primer, 200 µM of dATP, dCTP and dGTP, 150 µM of dTTP, 50 µM of biotin-16-dUTP, 2.5 U of Taq DNA polymerase (Boehringer Mannheim, Allemagne), 1 U of Uracil-DNA-glycosylase heat labile (Boehringer Mannheim, Allemagne), 1 ng of plasmid containing gyrase gene. Samples were first denatured at 94°C for 5 min. Then 40 cycles of amplification were performed consisting of 30 sec at 94°C, 45 sec at 48 °C and 30 sec at 72°C and a final extension step of 10 min at 72°C. Water controls were used as negative controls of the amplification. The sizes of the amplicons obtained using these primers were 166 bp for all genera.

Production of the capture nucleotide sequences and of the targets

[0436] The capture nucleotide sequences contain a spacer fixed on the support by its 5' end and of the following sequence 5'ATAAAAAAGTGGGTCTTAGAAATAAATTTCGAAGTGCAATAATTATTATTCAC AACATTTCGATTTTTGCAACTACTTCAGTTCACTCCA3') (SEQ ID NO: 209), followed by the following specific sequences for the various Gyrase from the different bacteria:

Name	Sequence (5' -> 3')
Capture	

nucleotide sequence	
A. Genus level	
T Staphy genus	GACTCWTCAATTTATGAWGCHATGGTAHGAAYGG (SEQ ID NO: 210)
T Entero genus	GACAGTGCGATYTAYGARTCAATGGTRCGG (SEQ ID NO: 211)
T Strepto genus	TGGTTCGTATGGCTCAATGGTGGAGYTAY (SEQ ID NO: 212)
B. Species level	
T S. aureus	CTCAAGATTTCAGTTATCGTTATCCGCT (SEQ ID NO: 213)
T S. epidermidis	CCCAAGACTTTAGTTATCGTTATCCACT (SEQ ID NO: 214)
T S. hominis	CACAAACCTTTAGCTATCGTTATCCTC (SEQ ID NO: 215)
T Entero.faecium	ACAGCCATTCAGCTACCGTTATATGCT (SEQ ID NO: 216)
T Entero.faecalis	AACCTTTTAGTTATCGGGCTATGTTAGTT (SEQ ID NO: 217)
T S. pneumoniae	GATGGAGATAGTGCTGCCGCTCAAC (SEQ ID NO: 218)
T S. epyogenes	CTTGTTGATGGGCATGGCAATTTTGG (SEQ ID NO: 219)
T H. influenzae	TTCTCACTTCGCTATATGTTGGTTGATG (SEQ ID NO: 220)

[0437] The capture nucleotide sequences were first synthesized chemically and later on produced by PCR amplification after cloning of the sequences into the plasmid

pGEM-T Easy Vector System(Promega, Madison, USA). The capture nucleotide sequences were then produced by amplification of the plasmids using a common 5' aminated primer 5' GAATTCAAAGTTGCTGAGAATAGTTCA (SEQ ID NO: 221) and a second primer of 27 bases complementary of each capture nucleotide sequence.

[0438] The aminated capture polynucleotide sequences (longer than 100 bases) were spotted from solutions at concentrations ranging from 150 to 3000 nM. The capture nucleotide sequences were printed onto the aldehyde microscopic slides with a home made robotic device (250 µm pins from Genetix (UK). The solutions of spotting were from AAT (Namur, Belgium). The spots have 400 µm in diameter and the volume dispensed is about 0.5 nl. Slides are dried at room temperature and stored at 4 °C until used.

Hybridization

[0439] At 65 µl of hybridization solution (AAT, Namur, Belgium) were added 5 µl of amplicons and the solution was loaded on the array framed by an hybridization chamber. For positive controls 2 nM biotinylated CMV amplicons of 437 bp were added to the solution; their corresponding capture nucleotide sequences were spotted on the array. The chamber was closed with a coverslip and slides were denatured at 95 °C for 5 min. The hybridization was carried out at 65° for 30 min. Samples were then washed 4 times with a washing buffer.

Colorimetric detection

[0440] The glass samples were incubated 45 min at room temperature with 800 µl of streptavidin labelled with colloidal gold 1000 x diluted in blocking buffer (Maleic buffer 100 mM pH 7.5, NaCl 150 mM, Gloria milk powder 0.1%). After 5 washes with washing buffer, the presence of gold served for catalysis of silver reduction using a staining solution (Silver Blue Solution, AAT, Namur, Belgium). The slides were incubated 10 min with 800 µl of revelation mixture, then rinsed with water, dried and analyzed using a microarray reader (Worstation, AAT, Namur, Belgium). The spots of the arrays were then quantified by a specific quantification software.

Example 17: Detection of virus species and subtypes.

[0441] The virus to be detected were the adenovirus, the herpes virus 1, 5 and 4. The consensus primers for the virus amplification were A(G)C(A,T)G(C,T)GCCGCCGTGT(A)T(A,C)C(T)G(A,C) (SEQ ID NO: 222) and GT(G,C)G(T,A)GTTGTTTTTG(A)T(C)G(C)G(T) (SEQ ID NO: 223).

[0442] The amplicons of the virus are respectively of 315, 331, 779, and 820 bases long for the 4 virus corresponding to the sequences N°420-734, 7924-8254, 1562-2340, 120761-130580.

[0443] The conditions for the PCR amplification were as described in example 1 but with an annealing temperature of 45 °C. After amplification, the amplicons were hybridized on an array bearing the capture nucleotide sequences for each virus species and subtypes. The capture nucleotide sequences were composed of a spacer fixed by its 5' end to the slides and have the sequence as in example 16 and a specific part located on the 3' end of the capture nucleotide sequence.

[0444] Specific sequences of the capture nucleotide sequences:

[0445] Adenovirus : 5'-AACTCTTCTCGCTGGCACTCAAGAGTG-3' (SEQ ID NO: 224)

[0446] Herpes virus 1: 5'-GTGGAAGTCCTGATACCCATCCTACAC-3' (SEQ ID NO: 225)

[0447] Herpes virus 5: 5'-AAAAGCGTGTGATCTGACCGAGGCGAA-3' (SEQ ID NO: 226)

[0448] Herpes virus 4: 5'-AGGTCCTTGAGGAAGAAGTGTTCCAGG-3' (SEQ ID NO: 227)

[0449] Tm = 82°C

[0450] The hybridization, the colorimetry labelling and the quantification were performed as in example 1.

Example 18: Detection of cytochrome b sequences on array bearing species specific capture nucleotide sequences Example of meat origin

[0451] The amplified target sequences are fragments of the cytochrome b gene sequences corresponding to the different species were amplified by a PCR using the following consensus primers

[0452] Meat1 5' TCCTCCCATGAGGAGAAATAT 3' (SEQ ID NO: 228)

[0453] Meat2 5' AGCGAAGAATCGGGTAAGGGT 3' (SEQ ID NO: 229)

[0454] The PCR were performed as in example 1. The sizes of the amplicons obtained using these primers were between 130 and 147 bp for all genus. After amplification, the amplicons were hybridized on an array bearing the capture nucleotide sequences for each species. The capture nucleotide sequences were composed of a spacer fixed by its 5' end to the slides and having the same sequence as in example 1 and a specific part located on the 3' end of the capture nucleotide sequence.

[0455] *Spacer*
5'ATAAAAAAGTGGGTCTTAGAAATAAATTTCGAAGTGCAATAATTATTATTCAC AACATTTCGATTTTTGCAACTACTTCAGTTCACTCCA3' (SEQ ID NO: 209)

[0456] Specific sequences of the capture nucleotide sequences

[0457] Chicken
CCTTAACGACTCTTATCCAAACACTATGCCACCGGGGAG (SEQ ID NO: 230)

[0458] Duck CCCTAACGACTCTTATCCAAACACTACTGCCATCGGGGAG (SEQ ID NO: 231)

[0459] Ostrich CCTTAACGAACTCTAAG (SEQ ID NO: 232)

[0460] Pig AAAGAGGAGTAGAATCACGATTAAG (SEQ ID NO: 233)

[0461] Quail CCATGTCGACTCTTATCCAAACACTACTGCCATCGTGGAG (SEQ ID NO: 234)

[0462] Rabbit
CCCTAACGACTATCCTCCAATCACTAATGCCAACGAGGGG (SEQ ID NO: 235)

[0463] Turkey CCCTAACGACTCTTATCCAAACACTACTGCCATCGGGAG (SEQ ID NO: 236)

[0464] Wildpig CCCTATCGACTATCTTCTAAACACTACTGGCATCGAGGAG (SEQ ID NO: 237)

[0465] Cow CCTAACGACTATTCTCCAACCACTACTGACAACGAGGAG (SEQ ID NO: 238)

[0466] The consensus capture nucleotide sequence for all these animal detection is

[0467]

ATTCTGAGGGGCACCGTCATCACAAACCTATTTCAGCAATCCCC TACATGGCAAACCCTAGTAGAATGAGCCTGAGGGGGATTTTCAGTGACAACC (SEQ ID NO: 239)

[0468] To identify the cow species, another couple of consensus primer was design

[0469] Cow1 AAGACATAATATGTATATAGTAC (SEQ ID NO: 240)

[0470] Cow2 GAAAAATTTAAATAAGTATCTAG (SEQ ID NO: 241)

[0471] Specific capture nucleotide sequences have been designed

[0472] BrownSwiss GCGGCATGATAATTA (SEQ ID NO: 242)

[0473] Jersey CGCTATTCAATGAAT (SEQ ID NO: 243)

[0474] Ayrshire GCTCACCATAACTGT (SEQ ID NO: 244)

[0475] Hereford ATCTGATGGTAAGGA (SEQ ID NO: 245)

[0476] Simmental ATAAGCCTGGACATT (SEQ ID NO: 246)

[0477] Piemontaise ATAAGCATGGACATT (SEQ ID NO: 247)

[0478] Canadienne TCACTCGGCATGATA (SEQ ID NO: 248)

[0479] RedAngus AATGGTAGGGGATAT (SEQ ID NO: 249)

[0480] Limousine ATGGACTCATGGCTA (SEQ ID NO: 250)

[0481] AberdeenAngus TATTCAATGAACTTT (SEQ ID NO: 251)

[0482] Butana GCATGGGGTATATAA (SEQ ID NO: 252)

[0483] Charolais ATAAGCGTGGACATTA (SEQ ID NO: 253)

[0484] Fresian CCTTAAATACCTACC (SEQ ID NO: 254)

[0485] Kenana TGCTATAGAAGTCAT (SEQ ID NO: 255)

[0486] N'Dama TGTTATAGAAGTCAT (SEQ ID NO: 256)

[0487] The hybridization, the colorimetry labelling and the quantification were performed as in example 1.

Example 19: Detection of Sucrose synthase sequences on array bearing species specific capture nucleotide sequences Example of plant origin

[0488] The amplified targets are fragments of the sucrose synthase gene sequences corresponding to the different species were amplified by a PCR using the following consensus primers:

[0489] PPss3 5' GGTTTGGAGARRGGNTGGGG 3' (SEQ ID NO: 257)

[0490] PPss4 5' TCCAADATGTAVACAACCTG 3' (SEQ ID NO: 258)

[0491] The PCR were performed as in example 1. The sizes of the amplicons obtained using these primers were 221 bp for all genus. After amplification, the amplicons were hybridized on an array bearing the capture nucleotide sequences for each species . The capture nucleotide sequences were composed of a spacer fixed by its 5' end to the slides and having the following sequence and a specific part located on the 3' end of the capture nucleotide sequence.

[0492] *Spacer*

5'ATAAAAAAGTGGGTCTTAGAAATAAATTTCGAAGTGCAATAATTATTATTCAC AACATTTCGATTTTTGCAACTACTTCAGTTCACTCCA3' (SEQ ID NO: 209)

[0493] Specific sequences of the capture nucleotide sequences:

[0494] TPss1 (potato) GAAGCATGCATACCATCTCTAGCA (SEQ ID NO: 259)

[0495] TPss3 (tomato) GGAGCATGCAGATCATCTCTAGAA (SEQ ID NO: 260)

[0496] TPss7 (oryza) GAAGCAAGTGGATGGTGTCAAGCA (SEQ ID NO: 261)

[0497] TPss8 (zea) AGAGGAGGTGGATAGTCTCCTGTG (SEQ ID NO: 262)

[0498] TPss9 (soja) AGAGAAGTTGAATTGACTCAAGGA (SEQ ID NO: 263)

[0499] TPss11 (wheat) AGAGAAGGTGGATAGTCTCGCTCG (SEQ ID NO: 264)

[0500] TPss12 (bareley) AGAGAAGGTGGATAGTCTCGCTCG (SEQ ID NO: 265)

[0501] TPss13 (bean) ATAGAAGCTGAATGGACTCGAGCA (SEQ ID NO: 266)

[0502] TPss14 (carrot) GAAGCATGTGAAACATCTCAGTAA (SEQ ID NO: 267)

[0503] The hybridization, the colorimetry labelling and the quantification were performed as in example 1.

Example 20: Detection of Cytochrome b sequences on array bearing species specific capture nucleotide sequences Example of fishes species, genus and families

[0504] The amplified target sequences are fragments of the cytochrome b gene sequences corresponding to the different species were amplified by a PCR using the following consensus primers:

[0505] Fish1 5' ACTATTHCTAGCCATVCAYTA 3' (SEQ ID NO: 268)

[0506] Fish2 5' AGGTAGGAGCCATAAAGACCTCG 3' (SEQ ID NO: 269)

[0507] The PCR were performed as in example 1. The sizes of the amplicons obtained using these primers were 170 bp for all genus. After amplification, the amplicons were hybridized on an array bearing the capture nucleotide sequences for each species. The capture nucleotide sequences were composed of a spacer fixed by its 5' end to the slides and having the following sequence and a specific part located on the 3' end of the capture nucleotide sequence.

Spacer

[0508]

5'ATAAAAAAGTGGGTCTTAGAAATAAATTTCGAAGTGCAATAA TTATTATTCACAACATTTCGATTTTTGCAACTACTTCAGTTCACTCCA3' (SEQ ID NO: 209)

[0509] Specific sequences of the capture nucleotide sequences for the species:

[0510] G. morhua AAGGCTTAATCAGTCGGCATCAAATGTA (SEQ ID NO: 270)

[0511] G. macrocephalus AAGGCTTACTCAGTTGGCATTAAATGTA (SEQ ID NO: 271)

[0512] P. flesus GAAGCCTACTCAGTTGGCATCAACTGCA (SEQ ID NO: 272)

[0513] M. merluccius AACGCCTAATCAGTAGGCATTAAATGCA (SEQ ID NO: 273)

[0514] O. mykiss AAAGCTTACTCAGTCGGCATTGATTGTA (SEQ ID NO: 274)

[0515] P. platessa GAAGCCTATTCAGTCGGCATCAACTGCA (SEQ ID NO: 275)

[0516] P. virens AAAGCTTAATTAGTCGGCATTAAATGTA (SEQ ID NO: 276)

[0517] S. salar CAATGCCTACTCAGTCGGTATCGATTGTA (SEQ ID NO: 277)

[0518] S. pilchardus GAAGCTTAGTCAGTAGGCATCAAATGCA (SEQ ID NO: 278)

[0519] A. thazard AAAGCCTATTCAGTTGGCTTCAAATGTA (SEQ ID NO: 279)

[0520] T. alalunga AAAGCCTACTCAGTAGGCTTCAAATGTA (SEQ ID NO: 280)

[0521] T. obesus AAAGCCTACTCAGTTGGCTTTAACTGTTA (SEQ ID NO: 281)

[0522] R. hippoglossoides GAAGCCTATTCAGTCGGCATCAACTGCA (SEQ ID NO: 282)

[0523] S. trutta AAAGCCTACTCAGTCGGCATCGATTGCA (SEQ ID NO: 283)

[0524] S. sarda AAAGCCTAATCAGTCGGCTTTAATTGCA (SEQ ID NO: 284)

[0525] T. thynnus AAGGCCTATTCAGTTGGCTTCAACTGTA (SEQ ID NO: 285)

[0526] S. scombrus AACGCCTACTCAGTAGGCTTCAAATGCA (SEQ ID NO: 286)

[0527] Specific sequences of the capture nucleotide sequences for the families:

[0528] Salmonidae

[0529] AAACATTCACGCTAACGGAGCATCTTTCTTCTTTATCTGT (SEQ ID NO: 287)

[0530] Pleuronectidae

[0531] AAGCATTCATGCCAACGGCGCATCATTCTTTTTCATTTGC (SEQ ID NO: 288)

[0532] Pleuronectidae

[0533] GAATATACATGCTAATGGTGCCTCTTTCTTTTTTATTTGT (SEQ ID NO: 289)

[0534] Scombridae

[0535] AAACCTCCACGCAAACGGAGCCTCTTTCTTTCTTTATCTGC (SEQ ID NO: 290)

[0536] Among this family, a consensus capture nucleotide sequence was designed to detect the Thunnus genus

[0537] ATTCCACATCGGCCG (SEQ ID NO: 291)

[0538] Consensus capture nucleotide sequences for these various fish families:

[0539]

ATCCGAAACATCCACGCAACGGGCATCTTTCTTCTTTATCTGTA TCTACTTACACAT (SEQ ID NO: 292)

[0540] The hybridization, the colorimetry labelling and the quantification were performed as in example 1.

Example 21: Detection of Cytochrome P450 isoforms after amplification with consensus primers and hybridization of the amplicons on arrays.

[0541] The amplified targets are fragments of the cytochrome P450 gene sequences corresponding to the different families which were amplified by a PCR using the following consensus primers:

[0542] p450-1 5'TCCGCAACTTGGGCCTGGGCAAGA 3' (SEQ ID NO: 293)

[0543] p450-2 5'CCTTCTCCATCTCTGCCAGGAAG 3' (SEQ ID NO: 294)

[0544] The conditions for the PCR amplification are the same as in example 1. The sizes of the amplicons obtained using these primers were 970 bp. After amplification, the amplicons were hybridized on an array bearing the capture nucleotide sequences for each single point mutations.

[0545] The capture nucleotide sequences were composed of a spacer fixed by its 5' end to the slides and having the following sequence and a specific part located on the 3' end of the capture nucleotide sequence.

[0546] *Spacer* 5' GAATTCAAAGTTGCTGAGAATAGTTCAATGGAAGGAAGCG 3' (SEQ ID NO: 36)

[0547] Specific sequences of the capture nucleotide sequences for the single point mutations from different families of cytochrome p450.

- Target Gene : Human CYP2D6

Name		**Sequence (5'-3')**
WT		GAAAGGGGCGTCCTGGG (SEQ ID NO: 295)
***4**	substitution T in C at position 13 of WT	GAAAGGGGCGTCtTGGG (SEQ ID NO: 296)
WT		GCTAACTGAGCACAGGA (SEQ ID NO: 297)

***3**	Deletion of A at position 14 of WT	GCTAACTGAGCACGGA (SEQ ID NO: 298)
WT		CTCGGTCACCCCCTGC (SEQ ID NO: 299)
***6**	Deletion of C at position 12 of WT	CTCGGTCACCCCTGC (SEQ ID NO: 300)

- Target Gene : Human CYP2C19

Name		Sequence (5'-3')
WT		AATTATTTCCCAGGAA(SEQ ID NO: 301)
***2**	substitution G in A	AATTATTTCCCaGGAA (SEQ ID NO: 302)
WT		AGCACCCCCTGAATCC (SEQ ID NO: 303)
***3**	substitution G in A	AGCACCCCCTGaATCC (SEQ ID NO: 304)

[0548] The hybridization, the colorimetry labelling and the quantification were performed as in example 1.

Example 22: Evidence for bacterial presence during the PCR (Real Time PCR) and identification on microarrays.

[0549] Example of detection of the main bacteria responsible for meningitis by real-time PCR on cerebrospinal fluid was combined with genus and species sequence identification on DNA microarray

[0550] The *tuf* is phylogenetically well conserved gene amongst bacteria, it encodes an elongation factor (TE). The biological sample for the detection of meningitis was cerebrospinal fluid. Indeed, this medium is normally sterile and if there is an infection, it would be contaminated by only one pathogen. Thus it limits the risk to amplify other genus with consensus primers.

[0551] For a real-time PCR consensus primers for the *tuf* gene, amplify all genus and species of interest and the consensus probe for the *tuf* gene was labelled with two fluorochromes (quencher and emettor) as internal control of the PCR.

[0552] Biochips bearing specific capture probes for bacteria genus and species currently found in meningitis infections were:

[0553] *Neisseria menengitidis* serogroupA

[0554] *Neisseria menengitidis* serogroupB

[0555] *Haemophylus influenzae*

[0556] *Escherichia coli*

[0557] *Streptococcus pneumoniae*

[0558] *Streptococcus agalactiae*

[0559] *Staphylococcus aureus*

[0560] *Staphylococcus epidermidis*

[0561] *Staphylococcus haemolyticus*

[0562] *Staphylococcus hominis*

Staphylococcus saprophyticus

[0563] For the Primers Consensus Sense were:

[0564] 5' GAATTRGTTGAAATGGAA 3' 18 nt (SEQ ID NO: 305)

(R = A or G)

[0565] position 443-460 Tm = 46-48°C

[0566] 1 mismatch maximum

[0567] For the Consensus Antisense were:

[0568] 5' GTAGTACGGAARTAGAA 3' 17 nt (SEQ ID NO: 306)

(R = A or G)

[0569] position 995-1011 Tm = 46-48°C

[0570] 1 mismatch maximum

[0571] For the Double labelled Probe (sense) were:

[0572] 5' GGTGTTGAAATGTTCC 3' 16 nt (SEQ ID NO: 307)

[0573] position 776-792 Tm = 46°C

[0574] 1 mismatch maximum

[0575] Size of the amplified product : 569 bp

Genus specific capture probes

[0576] 1) Meningococcus 5' CGACCTGCTGTCCAGCT 3'(17 nt) (SEQ ID NO: 308)

[0577] Identical for serogroup A and B and a minimum of 5 mismatches against the other genus.

[0578] 2) Streptococcus 5' CTTCAGGACGTATCGACC 3'(18 nt) (SEQ ID NO: 309)

[0579] Identical for *Streptococcus pneumoniae* and *Streptococcus agalactiae* and a minimum of 5 mismatches against the other genus.

[0580] 3) Staphylococcus 5' TTATTAGACTACGCTGAAG 3'(19 nt) (SEQ ID NO: 310)

[0581] Identical for *Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Staphylococcus hominis, Staphylococcus saprophyticus* and a minimum of 6 mismatches against the other genus.

Species specific capture probes

[0582] 1) Neisseria menengitidis serogroup A

[0583] 5' TCTATTTCCGGTCGTGGT 3'(18 nt) (SEQ ID NO: 311)

[0584] 2) Neisseria menengitidis serogroup B

[0585] 5' CCATTTCCGGCCGCGG 3'(16 nt) (SEQ ID NO: 312)

[0586] 3) Haemophylus influenzae

[0587] 5' GAGTTAGCAAACCACTTAG 3'(19 nt) (SEQ ID NO: 313)

[0588] 4) Escherichia coli

[0589] 5' AACTGGCTGGCTTCCTG 3'(17 nt) (SEQ ID NO: 314)

[0590] 5) Streptococcus pneumoniae

[0591] 5' GTATCAAAGAAGAAACTCAAA 3'(21 nt) (SEQ ID NO: 315)

[0592] 6) Streptococcus agalactiae

[0593] 5' GTATTAAAGAAGATATCCAAA 3'(21 nt) (SEQ ID NO: 316)

[0594] 7) Staphylococcus aureus

[0595] 5' GGTTTACATGACACATCTAA 3'(20 nt) (SEQ ID NO: 317)

[0596] 8) Staphylococcus epidermidis

[0597] 5' GTATGCACGAAACTTCTAAA 3'(20 nt) (SEQ ID NO: 318)

[0598] 9) Staphylococcus haemolyticus

[0599] 5' GTATCCATGACACTTCTAAA 3'(20 nt) (SEQ ID NO: 319)

[0600] 10) Staphylococcus hominis

[0601] 5' GGTATCAAAGAAACTTCTAAA 3'(21 nt) (SEQ ID NO: 320)

[0602] 11) Staphylococcus saprophyticus

[0603] 5' ATGCAAGAAGAATCAAGCAA 3'(20 nt) (SEQ ID NO: 321)

[0604] Each of the sequences above comprised a spacer at its 5' end Spacer sequence 5' GAATTCAAAGTTGCTGAGAATAGTTCAATGGAAGGAAGCG 3' (SEQ ID NO: 36). Capture probes were aminated at their 5' end.

Example 23: HLA Identification

[0605] Glass surface was activated in order to bear aldehydes groups as proposed by EP-00870184.9. The slides were then incubated with a Protein A at 5 µg/ml in PBS solution for 60 min. The slides were washed in PBS and then incubated for 5 min. in

$NaBH_4$ solution at 2.5 mg/ml. After washing they were incubated for 2h with 10% milk powder and then washed again. Antibodies at concentration of 0.1 mg/ml were spotted on the glass slides with solid pins of 0.250 mm diameter and the spots were around 0.35 mm diameter final. The spotting solution contained buffer borate 0.05 M pH 8, glycerol 40% and NP40 0.02%. After 3 washes with 0.01 M phosphate pH 7.4, non-specific binding sites were blocked with PBS containing milk powder at 0.1% for 1h at 20°C.

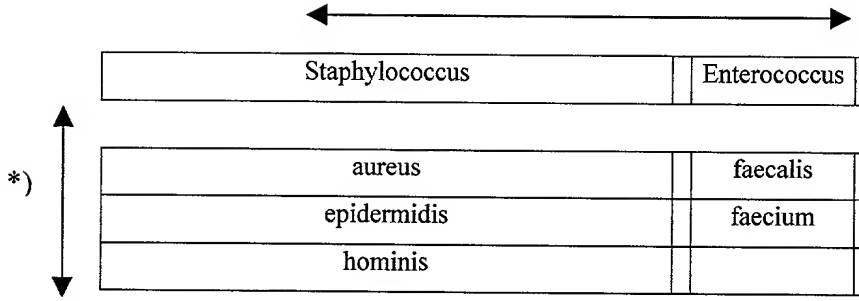
[0606] For the reaction of the targets, the slides were incubated for 1h at 20°C with the samples in the presence of PBS containing milk powder at 0.1%. After 4 washes of one minute with a 10 mM maleate buffer containing 15 mM NaCl (washing buffer) the slides were incubated for 45 min. at 20°C with an antibody common for the various targets potentially present in the samples, then with a conjugate of anti-IgG/gold particles of 10 nm diameter (diluted 100 times) in 100 mM maleate buffer containing 150 mM NaCl.

[0607] The slides were washed 5 times in the same washing buffer as before and then incubated for 10 min. in the Silver Blue detection solution (AAT Namur) for obtaining the silver crystal precipitation. The slides were finally washed in water before being read in the Silver Blue Reader (AAT).

[0608] The HLA-A typing was obtained using antibodies specific of the types or subtypes. The antibodies against HLA-ABC common, HLA-B7, HLA-B27, were obtained from Cymbus Biotechnology, Ltd., Hampshire, UK. Other antibodies were from Pel-Freez especially the antibodies directed against the HLA-A2, A203 and A210 or HLA-B39, B3901, B3902, which allow typing and subtyping of the HLA. Lymphocytes were isolated from the blood according to the classical microlypophocytotoxicity assay (Pel-Freez, Brown Deer, Wisconsin, USA). Lymphocytes at 10x106 cells/ml were incubated for 30 min. at 37°C with the antibody array in RPMI 1640 media with Hepes buffer. The arrays are then washed 4 times in the same medium. The second antibodies for cells were directed against CD-2 and CD-19. Then the anti-IgG/nano-gold complexes were incubated followed by the Silver Blue (AAT, Namur, Belgium) for the detection.

Table 1

Horizontal identification of the genus



	Staphylococcus	Enterococcus	Haemophilus
*)	aureus	faecalis	influenzae
	epidermidis	faecium	
	hominis		

(*) Identification of the species

Table 2

Meat

Galinacea	**Leporidae**	**Suidae**	**Bovidae**
Chicken	Rabbit	Pig	Cow
Duck		Wild pig	Brownswiss, Jersey, Hereford, Simmental, Piemontaise, Canadienne, RedAngus, Limousine, AberdeenAngus, Butana, Charolais, Fresian, Kenana, N'Dama
Ostrich			
Turkey			
Quail			

Table 3

Fish families

Classification						
– **Family**	**Scombridae**	**Salmonidae**	**Merlucciidae**	**Pleuronectidae**	**Gadidae**	**Clupeidae**
– Genera	Auxis	Oncorhynchus	Merluccius	Pleuronectes	Pollachius	Sardina
– Species	A. thazard	O. mykiss	M. merluccius	P. platessus	P. virens	S. pilchardus
– Genera	Sarda	Salmo		Platichthys	Gadus	
– Species	S. sarda	S.salar S.trutta		P. flesus	G.morhua G.macrocephalus	
– Genera	Scomber			Reinhardtius		
– Species	S. scombrus			R. hippoglossoides		
– Genera	Thunnus					
– Species	T.albacares T.obesus T.alalunga T.thynnus					

Animal Meat

Classification				
Family	**Galinacea**	**Leporidae**	**Suidae**	**Bovidae**
Genera	Chicken	Rabbit	Pig	
Genera	Duck		Wild pig	
Genera	Ostrich			
Genera	Turkey			
Genera	Quail			Cow
Species				Brownswiss, Jersey, Hereford, Simmental, Piemontaise, Canadienne, RedAngus, Limousine, AberdeenAngus, Butana, Charolais, Fresian, Kenana, N'Dama